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LESIONS INDUCED IN THE LUNGS BY INTRAVENOUS INJECTION OF TAR

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The extensive literature dealing with the production of tar carcinoma, which has been thoroughly reviewed by Woglom,[1] is concerned largely with painting the skin with tar. The carcinogenic effect of tar introduced into specific organs has not been so extensively investigated.

Production of carcinoma in the various internal organs after application of tar has been reported as follows: in rabbits, carcinoma of the breast (Yamagiwa and Ichikawa [2]) in thirty-three months, of the stomach and intestines (Ishibashi and Ohtani [3]) in two weeks and of the rectum (Leitch [4]); in rats, carcinoma of the bladder (Maisin and Picard [5]) in one hundred and fifteen days, of the stomach (Menetrier [6]) in three hundred and forty-one days and of the uterus (Teutschlaender [7]) in two hundred and forty-two days; in guinea-pigs, carcinoma of the gallbladder (Leitch [8] and Kazama [9]) in from five and one-half to twelve months.

Attempts have been made by a number of investigators to induce carcinoma of the lungs by the introduction of tar into these organs by various methods. The results of these experiments have been sufficiently confusing, both as to the actual production of carcinoma and as to the part played by the tar, to warrant the repetition of the procedure.

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1. Woglom, W. H.: Arch. Path. **2**:533 and 709, 1926.
2. Yamagiwa, K., and Ichikawa, K.: Mitt. a. d. med. Fakult. d. k. Univ. zu Tokyo **22**:1, 1919; Japan M. World, June 15, 1919; abstr., J. A. M. A. **73**:298, 1919.
3. Ishibashi, M., and Ohtani, S.: Gann **15**:2, 1921.
4. Leitch, A.: Compt. rend. Cong. du cancer, 1923, p. 39.
5. Maisin, J., and Picard, E.: Compt. rend. Soc. de biol. **91**:799, 1924.
6. Menetrier, P.: Bull. Assoc. franç. p. l'étude du cancer **13**:563, 1924.
7. Teutschlaender, M.: Ztschr. f. Krebsforsch. **23**:161, 1926.
8. Leitch, A.: Brit. M. J. **2**:451, 1924.
9. Kazama, Y.: Gann **16**:14, 1922; Japan M. World **4**:277, 1924.

Ibuka [10] observed atypical epithelial growths in the lungs of three rabbits in from fifteen to sixty-eight days after introducing blocks of paraffin mixed with soot directly into these organs. Kimura [11] placed coal tar in the bronchi of rabbits and induced in eighty days an adenoma-like growth, which consisted of a small encapsulated area composed of tortuous tubules lined with a single layer of epithelium. In the same series, an animal that lived one hundred and forty days was reported to have had carcinoma of the lung. Nakano [12] gave rabbits by intravenous injection from 0.2 to 0.4 cc. of a mixture of hydrous wool fat and tar. In eight, or approximately 9 per cent, of the animals which survived ten days or longer there developed carcinoma of the lungs. Two of the lesions were described as "typical adenocancroid" and six as "beginning carcinoma." Less marked "adenomatous hyperplasia" and metaplasia were observed in almost 70 per cent of the animals. Nakano expressed the belief that only the bronchial epithelium took part in the proliferation. These changes were associated with areas of necrosis induced by oil emboli. No mention is made of metastases in any case. Tedeschi [13] injected from 2 to 3 drops of tar twice a week into a superficial vein of the ear of seven rabbits. These animals survived for from a few hours to eighty days after the first injection. The epithelium of the smaller bronchi of some of these animals showed hyperplasia with atypical cells and nuclei, a change which Tedeschi believed represented a precancerous condition. Garschin and Pigalew [14] injected 0.1 cc. of Russian tar directly into a lung of each of twenty-three rabbits and killed the animals at intervals varying from one to eighty days after the injection. In spite of the histologic resemblance of the resulting epithelial hyperplasia to malignant proliferation, the authors interpreted the changes as regenerative and not as malignant.

Although the skin of the rabbit is known to be susceptible to the carcinogenic action of tar, it does not appear from the scattered references in the literature that unquestionable carcinoma of the lungs has been induced in rabbits by the introduction of tar into these organs by way of the blood stream or the bronchi or directly into the parenchyma. No one appears to have reported metastases. The longest duration of any of the experiments just cited was one hundred and fifty-nine days (Nakano [12]). In most of the experiments the time was too short for the development of true cancer at the site of the chronic irritation induced by the tar. Yamagiwa and Ichikawa [15] concluded that one hundred and

10. Ibuka, K.: Mitt. a. d. med. Fakult. d. k. Univ. zu Tokyo **30**:35, 1923.

11. Kimura, N.: Japan M. World **3**:45, 1923.

12. Nakano, M.: Mitt. a. d. med. Akad. zu Kioto **4**:142, 1930 (summary in German).

13. Tedeschi, C.: Tumori **16**:101, 1930.

14. Garschin, W. G., and Pigalew, J. A.: Ztschr. f. Krebsforsch. **33**:631, 1931.

15. Yamawiga, K., and Ichikawa, K.: J. Cancer Research **3**:1, 1918.

fifty or more days were required to produce tar cancer of the skin in rabbits, although they reported a few successful results in fifty-five days. Woglom [1] concluded from a study of the literature that "a period exceeding 150 days will usually be required for the production of a malignant new growth."

Rabbits were chosen for these experiments because they are susceptible to the carcinogenic action of tar and because spontaneous tumor of the lung in these animals is extremely rare. Polson [16] noted no primary tumor of the lung among sixty-six spontaneous tumors occurring in rabbits. Schmorl,[17] in 1903, merely mentioned carcinoma simplex in the lung of a rabbit. Jaffé [18] referred only to the observation of Schmorl and that of Petit, reference to which was not available to us.

METHODS

In our experiments, 5 Gm. of tar residue, prepared by the method described by Murphy and Landsteiner,[19] was added to 95 cc. of heavy liquid petrolatum, and the mixture was heated to just below the boiling point for from ten to twenty minutes. The mixture was allowed to cool gradually and after being passed through a cloth filter was ready for use. The tar used in these experiments was proved to be carcinogenic by the production of carcinoma on the backs of mice painted with it.

Thirty-two well nourished, apparently healthy rabbits of both sexes, chiefly rufous reds, were used in the experiments. Their weights ranged from 2,500 to 5,000 Gm. Depending on the constitutional and local reactions to the first injection, each animal was given an injection of 0.2 or 0.4 cc. of the tar-oil mixture slowly into a vein of the ear, usually once a week. Considerable difficulty was encountered with later injections because of the obliteration of the vein by thrombosis. If an animal became undernourished and appeared sick, the injections were discontinued. One animal received one injection of 1 cc. of anthracene intravenously.

The rabbits were closely observed as to general health. All animals died spontaneously. Postmortem examination was made as soon after death as possible. The lungs were immediately removed and separated from the heart; the trachea and bronchi were opened, and the entire specimen was hardened in a dilute solution of formaldehyde, U. S. P. (1:10) for forty-eight hours or longer. The fixed mass was cut transversely in thin sections and carefully examined for macroscopic evidence of carcinoma. Sections taken from different portions of the lungs and peribronchial tissues were stained in the routine way with hematoxylin and eosin for microscopic examination.

OBSERVATIONS

Symptoms.—All the animals refused to eat for a day or two following an injection. The majority of the rabbits, in addition, showed signs of respiratory difficulty and sneezing. A few days before death a serosanguineous fluid escaped from

16. Polson, C. J.: J. Path. & Bact. **39**:603, 1927.

17. Schmorl, in discussion on Sternberg.[25]

18. Jaffé, R.: Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere, Berlin, Julius Springer, 1931, p. 728.

19. Murphy, J. B., and Landsteiner, K.: J. Exper. Med. **42**:809, 1925.

the nose and at times from the mouth. There was marked loss of weight in all animals that lived less than nine months, varying from 600 to 1,900 Gm., with an average of approximately 800 Gm. The decrease was proportionally greatest following the first injection.

Pathologic Changes.—On macroscopic examination the lesions noted in the lungs were chiefly bronchopneumonia, infarcts, hemorrhagic tracheitis and bronchitis, cavities thought to be due to dilatation of the terminal bronchioles and chronic pleurisy.

The chief interest pertained to the amount of epithelial proliferation induced in the lungs by injection of tar and oil. On this basis, the thirty-two animals may be divided into three groups.

Group 1.—These rabbits had no proliferation of either the bronchial or the alveolar epithelium. This group may be separated into subgroups A and B.

Subgroup A: Eight animals which had received total amounts of from 0.4 to 3 cc. of tar and oil in from two to nine injections died in from nine to thirty-six days after the first injection, or from one to three days after the last. In all these animals the lungs were intensely hyperemic, and many alveoli contained free blood while others were partially filled with desquamated epithelium. Polymorphonuclear leukocytes and lymphocytes were present in only moderate numbers. The epithelium of the bronchioles was extensively desquamated and in places was mixed with pus cells to form a solid mass that obliterated the lumen. Tar-oil emboli were abundant in the smaller blood vessels and interalveolar capillaries. The lumens of many small and medium-sized arteries and veins were completely obliterated either by masses of fibrin, leukocytes and oil droplets or by a growth of loose-meshed, edematous connective tissue. The connective tissue was of two types: In one type large mononuclear leukocytes containing brown, granular pigment were present in the occluding connective tissue. These growths were interpreted as organized thrombi. In the other type, there was no brown pigment, and the process appeared to be a proliferation of the connective tissue of the intima (endarteritis obliterans).

Subgroup B: Seven animals which had received from six to twelve injections of tar and oil survived the last injection for a year or more. Most of these rabbits died of acute bronchopneumonia. No scars or other chronic lesions traceable to the injections were observed in the lungs. There was no proliferation of bronchial or alveolar epithelium in the lungs of any rabbit in this subgroup.

Group 2.—These rabbits had very slight epithelial proliferation. Of the four animals in this group, three died early in the course of the experiment. These rabbits had received in from two to six injections a total amount of from 0.8 to 1.8 cc. of tar and oil; they died eight, nine and twenty-one days, respectively, after the first injection, or three, three and five days after the last. The lungs were extremely hyperemic and contained extensive hemorrhages into the alveoli and numerous tar-oil emboli in the arterioles and capillaries. Many blood vessels were thrombosed, and organization was in progress in some of the thrombi. In the marginal portions of the lungs of two of the animals were a few small areas of granulation tissue (organizing infarcts), along the proximal edges of which were three or four small spaces lined with low columnar epithelium.

The fourth animal in this group received nine injections of tar and oil, a total of 2.4 cc. The rabbit lived fourteen months after the last injection, or more than eighteen months after the first. In the peripheral portion of the lungs of this rabbit were a few small, moderately dense scars, within which were from three to eight small round, oval or slitlike spaces lined with tall columnar epithelium which showed no evidence of active growth. A few of these spaces contained small round, calcified masses.

GROUP 3.—These rabbits had marked epithelial proliferation with metaplasia. Of the thirteen animals in this group, twelve received injections of the tar-oil mixture and one of anthracene. The data pertaining to this group are presented in the table.

Group 3 may be roughly divided into three subgroups. The characteristics of each subgroup can best be illustrated by a description of the results obtained in one typical animal.

Subgroup A: The animals in this subgroup survived for from two to three and one-half weeks, except in one instance in which the rabbit (BL 5) lived seventeen weeks after the first injection but only twelve days after the last. In all the animals in this subgroup, acute changes in the lungs, such as hyperemia, hemorrhage and edema, dominated the microscopic picture. Even in rabbit BL 5 chronic changes were not prominent.

Data on Rabbits in the Group Showing Marked Epithelial Proliferation in the Lungs with Metaplasia Following Intravenous Injection of the Tar-Oil Mixture

Number of Injections	Total Amount Injected, Cc.	Period of Survival: Since Last Injection, Days	Period of Survival: Since First Injection, Days	Subgroup	Rabbit	Substance Injected
4	1.4	5	14	A	94	Tar and oil
6	1.8	0	16		49	Tar and oil
2	0.4	23	24		B 3	Tar and oil
2	0.4	23	24		B 4	Tar and oil
5	1.0	12	108		BL 5	Tar and oil
9	3.0	38	72	B	63	Tar and oil
2	0.4	24	25		B 2	Tar and oil
5	1.0	8	104	C	B 1 L	Tar and oil
5	1.0	9	105		BL 6	Tar and oil
6	1.2	237	277		O E	Tar and oil
5	1.0	8	104		B 1	Tar and oil
7	2.0	27	170		L R 3	Tar and oil
1	1.0	184	184		W R	Anthracene

Rabbit 49 received 1.8 cc. of the tar-oil mixture in six injections. It died during the last injection, sixteen days after the first injection. The lungs (with the trachea) weighed 52 Gm. and were dark red; in the base of each lung were several grayish nodules from 2 to 4 mm. in diameter. Several branches of the pulmonary artery contained adherent thrombi. The lining of the trachea and bronchi was intensely red. The lymph glands about the main bronchi were enlarged, soft and moist.

In sections tar-oil emboli were numerous. The capillaries of the alveolar walls and most of the patent arteries and veins were distended with blood, and many alveoli were filled with red blood cells, except in several places in which the histologic architecture of the lung was obscured by compact masses of large mononuclear and spindle-shaped cells and polymorphonuclear leukocytes. The gray nodules in the bases of the lower lobes were composed of areas of coagulative necrosis, sharply demarcated from the adjacent hyperemic lung tissue by a zone of edematous granulation tissue (beginning organization). In the granulation tissue were: (1) many irregularly shaped, often branching, masses of spindle-shaped epithelial cells with an occasional mitotic figure and (2) columnar cells arranged about a lumen. The alveoli immediately outside the zone of granulation tissue

were filled with desquamated epithelium and many large multinuclear giant cells. Scattered throughout the sections were several microscopic areas of granulation tissue containing irregular masses of spindle-shaped epithelial cells, some of which were extensions from the lining of adjacent bronchioles. Several bronchioles, especially in the vicinity of the areas of necrosis, had a stratified epithelial lining, the cells of the basal layer being cuboid and those of the surface layer, columnar. Many blood vessels contained organizing thrombi. In some of the branches of the pulmonary artery the intima was markedly thickened by increase of fibrous tissue and the lumen narrowed. The enlarged lymph glands showed only acute lymphadenitis.

In subgroup A there was a moderate degree of epithelial proliferation associated with the granulation tissue about areas of necrosis and accompanied by metaplasia in the form of masses of atypical squamous epithelial cells, some of which contained nuclei in mitosis. These changes were widely disseminated throughout both lungs. This dissemination and the constant association with an inflammatory reaction (granulation tissue) seems to warrant the conclusion that the changes represent inflammatory hyperplasia rather than a neoplastic process.

Subgroup B: This group contained two animals that survived the injections of the tar-oil mixture for a longer period than those of the preceding subgroup, with the one exception already mentioned. In these rabbits chronic changes in the lungs predominated. The epithelial proliferation was more marked than that in subgroup A and was somewhat different in type.

Rabbit 63 received 3 cc. of the tar-oil mixture in nine injections and died seventy-two days after the first injection, or thirty-eight days after the last. The lungs weighed 67 Gm. and were hyperemic and edematous. Fibrinopurulent pleurisy was present. There were areas of necrosis and of bronchopneumonia in the lower lobe of each lung. A small solitary abscess filled with thick, almost cheesy, white pus occurred in the lower lobe of the right lung. The lining of the trachea and bronchi was dusky red. The peribronchial lymph glands were enlarged, soft and moist.

In sections the pleura was thickened, fibrous, edematous and covered with fibrinopurulent exudate. Beneath the thickened pleura many alveoli were lined with cuboid epithelium, and many contained desquamated epithelial cells and polymorphonuclear leukocytes. The alveolar walls in this region were thickened and fibrous. Extensive atelectasis was present in all sections. Scattered throughout the sections were numerous groups of alveoli lined by cuboid epithelium and filled with desquamated epithelium and multinuclear giant cells. The bronchioles were filled with desquamated epithelium and pus cells, and many were completely denuded of the lining cells. In the areas of necrosis dim outlines of exudate-filled alveoli and bronchioles were still visible. Along the margin of these areas was a zone of granulation tissue more mature than in subgroup A. In this zone of vascular connective tissue were many spaces lined with either columnar or cuboid epithelium and filled with exudate in varying stages of necrosis. Between these spaces were numerous irregularly shaped, often branching, solid masses of squamous epithelium (fig. 1). Many of these masses were connected by thin extensions with the epithelium lining the near-by spaces. The lumens of many blood vessels were completely occluded by loose-meshed connective tissue. In some vessels this occluding connective tissue contained brown, granular pigment (hemosiderin), usually lying within a large mononuclear cell (organized thrombus); in other vessels hemosiderin was not observed (probably endarteritis obliterans). The peribronchial lymph glands showed only hyperplastic lymphadenitis.

In subgroup B the epithelial proliferation was more marked than in subgroup A. While metaplasia was present, there was a greater tendency toward the production of cuboid and columnar epithelium, which was arranged about the lumens (figs. 1 and 2).

Subgroup C: This group contained six animals that had received from five to seven injections of the tar-oil mixture and one animal that had received one injection of anthracene. These rabbits survived the last injection for periods varying from eight to two hundred and fifty-seven days and the first injection for from

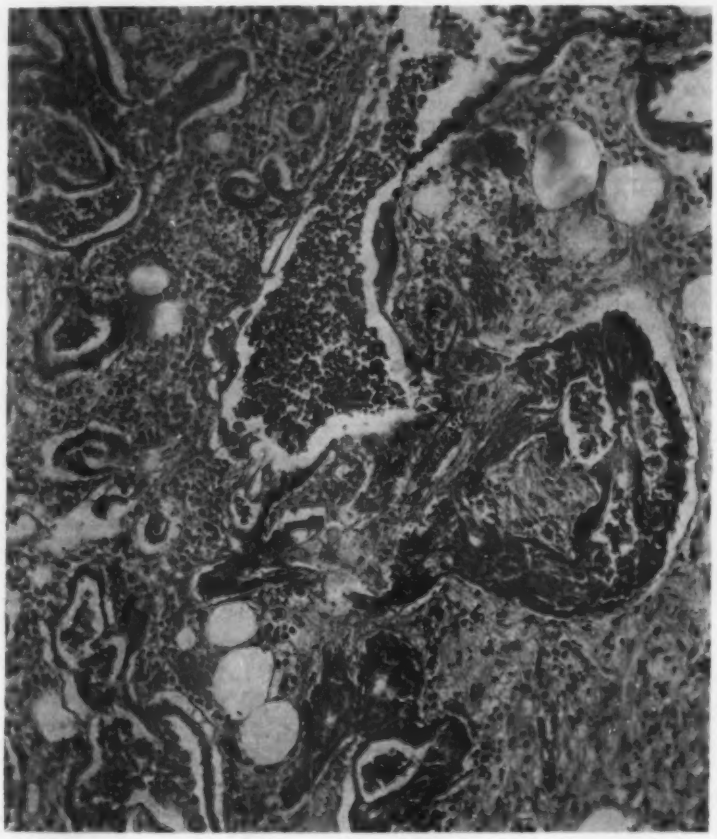

Fig. 1.—Atypical metaplastic epithelium in the lung of a rabbit that received three injections of 0.2 cc. each of the tar-oil mixture and died thirty-eight days after the last injection, or seventy-two days after the first. Numerous pus-filled cavities lined with different types of epithelium are shown and were present throughout the section. Magnification, × 125.

one hundred and four to two hundred and seventy-seven days. The lungs showed not only marked proliferation of the epithelium but also the formation of large cavities in the lungs that were first thought to be due to bronchiectasis.

Rabbit L R 3 received 2 cc. of the tar-oil mixture in seven injections and died twenty-seven days after the last injection, or one hundred and seventy days after

the first. The lungs weighed 102 Gm. Fibrinopurulent pleurisy with fibrous adhesions was noted between the lungs and the diaphragm. Patchy consolidation (bronchopneumonia) occurred in the lower lobe of each lung. Two small, encapsulated abscesses were present in the lower lobe of the right lung. Emphysema occurred in the upper lobe of each lung. The peribronchial lymph glands were enlarged.

Sections from one lung revealed a large area of necrosis surrounded by a capsule of connective tissue. On one side the necrotic material was partially absorbed, leaving a narrow space one wall of which was composed of necrotic tissue and the other of the capsule of the connective tissue, which was covered in

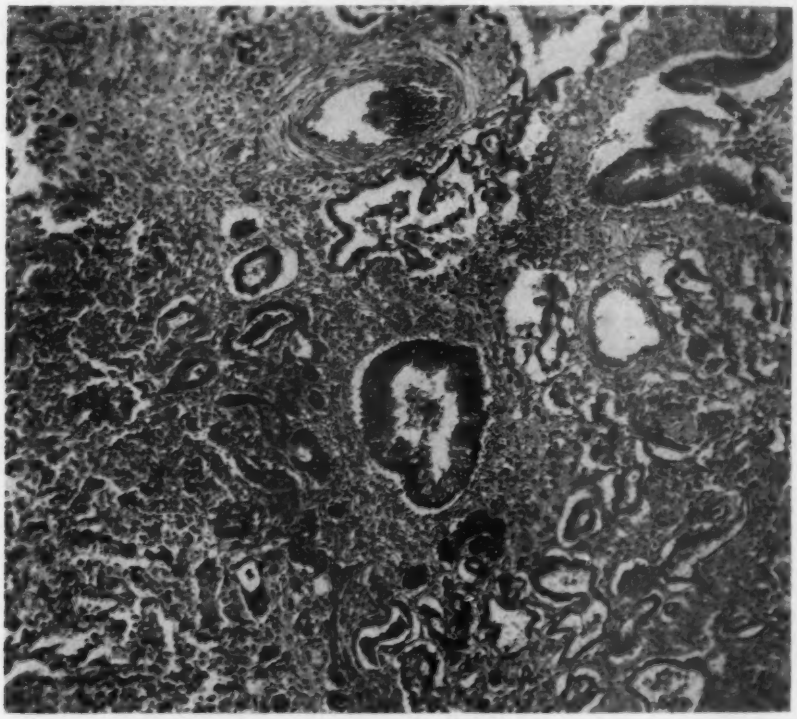

Fig. 2.—Small masses of metaplastic epithelium and extensive proliferation of bronchial and alveolar epithelium in the lung of a rabbit that received five injections of 0.2 cc. each of the tar-oil mixture and died one hundred and five days after the first injection. Magnification, × 95.

part in this region by a single layer of cuboid or columnar epithelium and in part by several layers of epithelial cells (fig. 4). In the substance of the capsule adjacent to this epithelial lining and in the near-by lung tissue cuboid and columnar cells lined small, round or oval spaces, most of which contained necrotic material. The epithelium lining some of these smaller spaces was continuous with that lining the narrow space next the necrotic mass. In the connective tissue between these small spaces were scattered, irregular masses of squamous cells. The connective tissue surrounding the large necrotic area on all sides, except on that

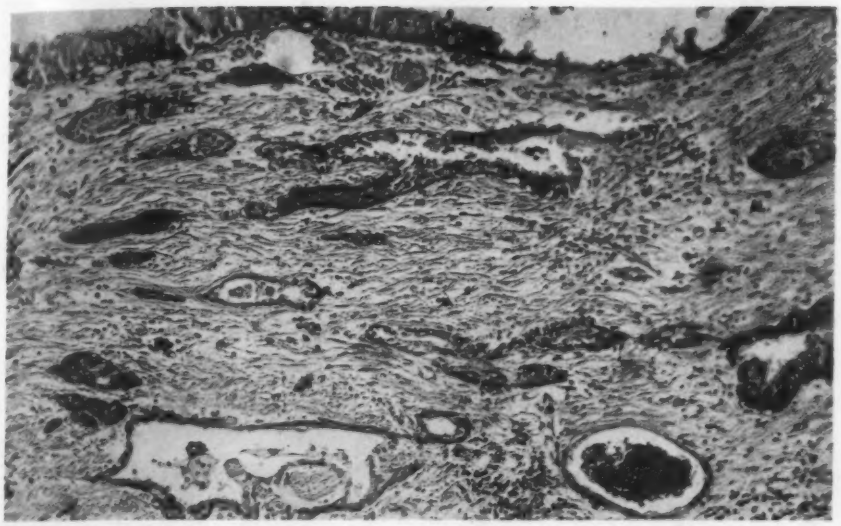

Fig. 3.—Metaplastic epithelium in the fibrous wall of a large cavity in the lung of a rabbit that received five injections and died one hundred and four days after the first injection.

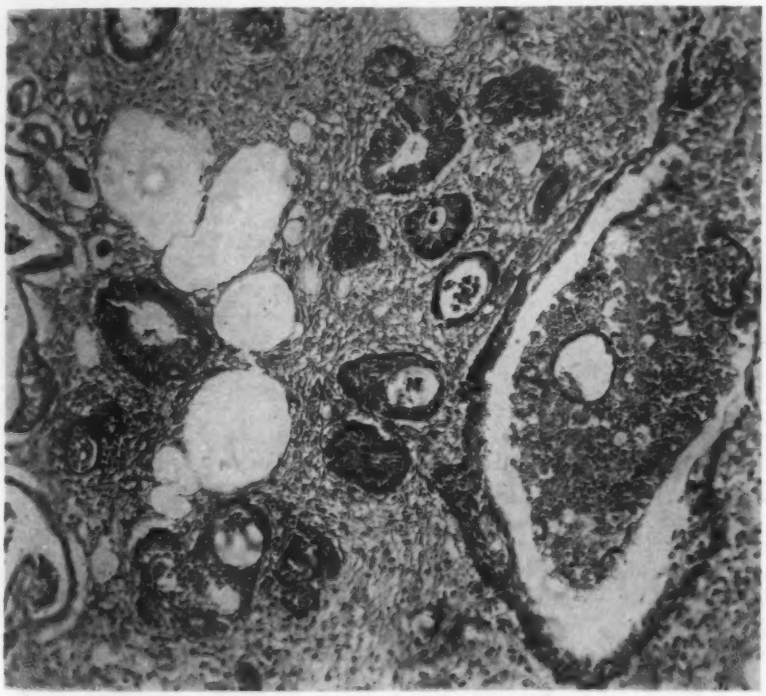

Fig. 4.—Early stage of cavity formation in a rabbit that received two injections of 0.2 cc. each and died twenty-four days after the first injection. The text should be consulted for the mechanism. Magnification, × 95.

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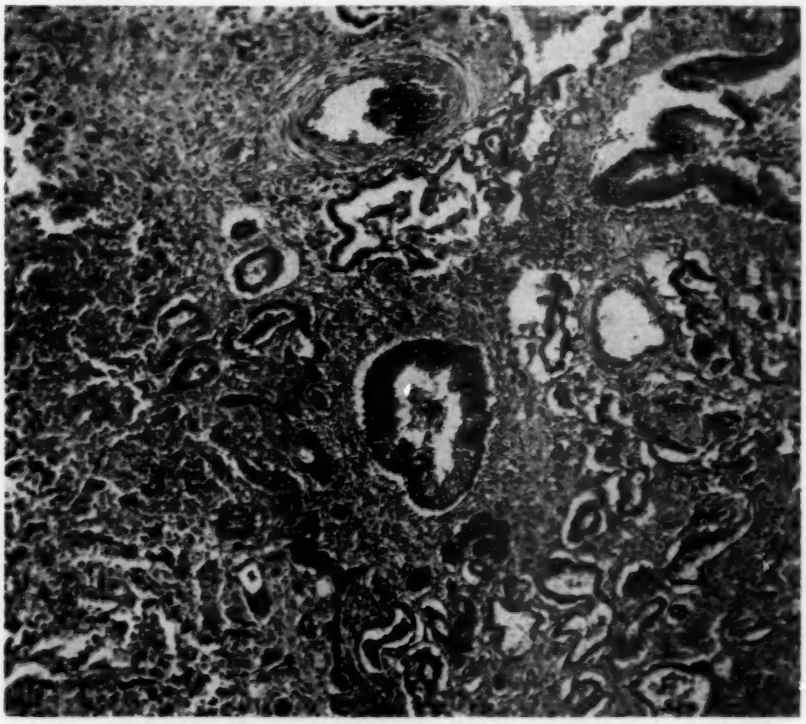

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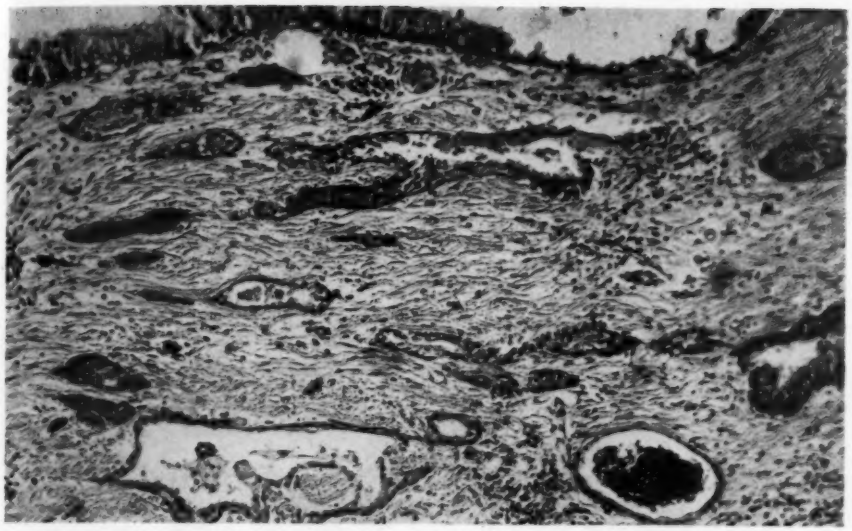

Fig. 3.—Metaplastic epithelium in the fibrous wall of a large cavity in the lung of a rabbit that received five injections and died one hundred and four days after the first injection.

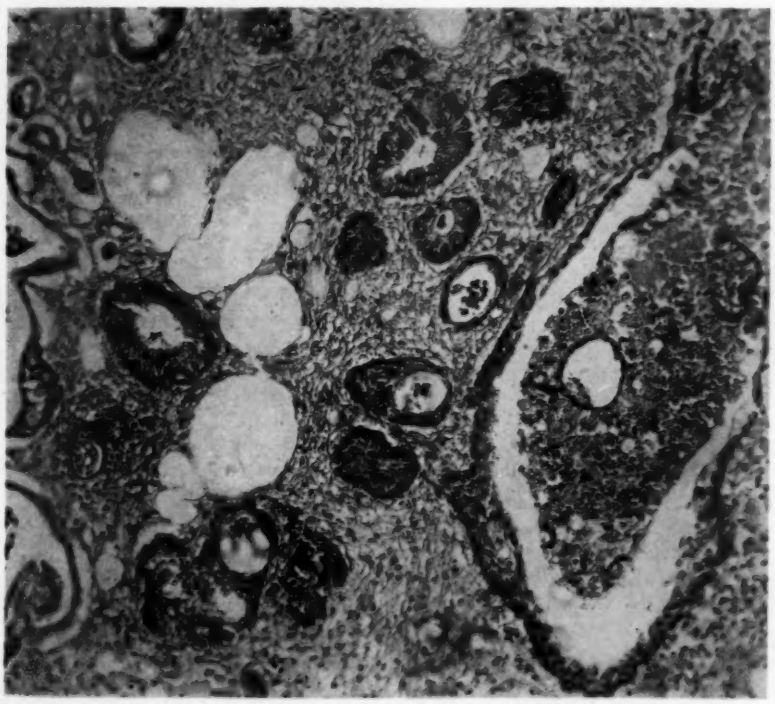

Fig. 4.—Early stage of cavity formation in a rabbit that received two injections of 0.2 cc. each and died twenty-four days after the first injection. The text should be consulted for the mechanism. Magnification, × 95.

next the narrow space, was free from epithelium, and the process of organization was proceeding in the usual way. In another section an area of necrosis showed no evidence of absorption, but in its capsule of connective tissue, except on the side next the pleura, there was much epithelial proliferation similar to that already described, namely, spaces lined with cuboid or columnar epithelium and masses of squamous cells.

This rabbit (L R 3) presented an early stage of a characteristic change observed in the lungs of all members of this subgroup, namely, large cavities surrounded by granulation and scar tissue and lined with epithelium (fig. 5). In the capsule of each of these cavities were many spaces lined with cuboid or columnar epithelium

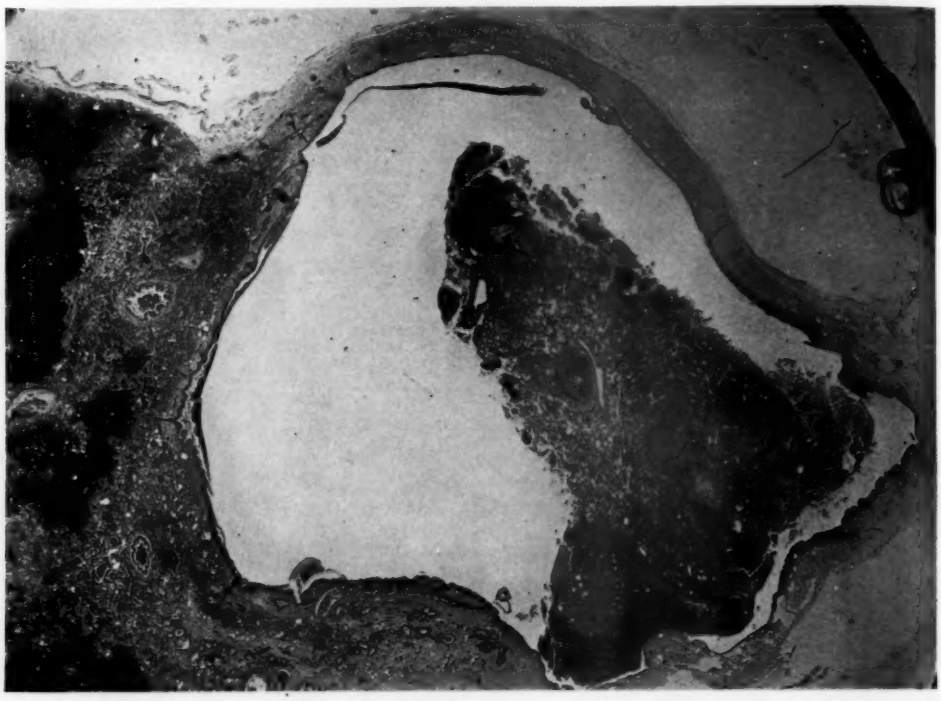

Fig. 5.—Late stage of cavity formation. The thickened pleura is seen in the upper part of the field and epithelial proliferation in the lower part and to the left. This section is from the animal from which was taken the tissue shown in figure 2.

and solid, irregular masses of spindle cells (figs. 3 and 5). In places connections could be traced between the epithelial structures in the capsule and the lining of the cavity. The walls of these cavities presented frequent papillary projections into the lumens. Solid masses of necrotic tissue lay free in some of the cavities. Since some of the necrotic masses were in process of organization and were partially covered with a single layer of low columnar epithelium or by squamous cells resting on granulation tissue, it is probable that they were attached to the wall of the cavity at some place other than that through which the section was made. The walls of a few of the cavities contained plates of cartilage. These probably represented true bronchiectatic cavities. The large majority did not contain car-

tilage. The lungs of all the animals in this subgroup presented widespread acute and chronic bronchopneumonia with extensive organization of the intra-alveolar exudate. The lumens of many blood vessels were completely obliterated from organization of thrombi or as a result of endarteritis obliterans.

COMMENT

Repeated intravenous injections of mixture of tar and liquid petrolatum in rabbits resulted in the following pathologic changes in the lungs:

In animals that died in two weeks or less after the first injection there were only marked hyperemia, hemorrhage, thrombosis and tar-oil emboli.

Several rabbits that survived the last injection for six or more months showed no results from the injections except an occasional obliterated blood vessel.

The lungs of seventeen of the thirty-two rabbits exhibited varying degrees of epithelial proliferation and chronic pathologic processes that may be summarized as follows: (1) permanent obliteration of the lumens of many blood vessels from organization of thrombi or from endarteritis obliterans; (2) multiple infarcts with subacute and chronic infection; (3) fibrous encapsulation of the necrotic areas and organization of the intra-alveolar exudate; (4) marked proliferation of the bronchial and alveolar epithelium about the encapsulated necrotic areas; (5) formation of large cavities lined with epithelium, and (6) enlarged peribronchial lymph glands which were the site of acute and chronic lymphadenitis without, in any case, the occurrence of metastases.

Some of these changes are readily explained. Two distinct processes appear to have been concerned in the obliteration of the lumens of blood vessels. In some instances, thrombi in various stages of organization were visible, and even in completely obliterated vessels hemosiderin was present in the mass of loose connective tissue which occupied the site of the lumen. In others, there appeared to be circumferential fibrous thickening of the intima and gradual narrowing of the lumen, which still contained blood, or complete obliteration of the lumen by fibrous tissue free from hemosiderin. The formation of infarcts followed the vascular changes, and their encapsulation and partial organization were the natural sequence.

The epithelium of the rabbit's lung appears to take part in the process of walling-off of masses of necrotic tissue. In sections from the lung of rabbit L R 3, already described, a necrotic mass was completely surrounded by a capsule of connective tissue. This capsule was closely adherent to the necrotic mass except at one place in which a narrow slit separated them. The inner surface of the capsule at this point was covered with epithelium. In other animals of group 3 large

cavities were completely lined with epithelium, and some of these contained free-lying necrotic masses, also partially covered with epithelium (fig. 5). Fischer [20] has reported similar changes in the lungs of rabbits receiving intravenous injections of "granuga oil." He expressed the belief that these cavities resulted from absorption of the necrotic material, the introduction of air through ulcerated bronchi and the subsequent lining of the cavity by the growth of epithelium over the inner surface of the capsule of connective tissue. He expressed the opinion that they did not result from bronchiectasis. This appears to be a satisfactory explanation of the origin of some of the cavities observed in the lungs of our rabbits. But other cavities in the lungs of these animals contained plates of cartilage in the wall. These were probably true bronchiectatic cavities. The walls of the cavities of either type exhibited marked epithelial proliferation.

Outside these cavities the epithelial proliferation, which in many animals was so extensive and atypical as to resemble a malignant neoplasm, bore no constant relation to the number of injections of tar and oil, the total amount injected or the length of time of survival after the last injection. None of the rabbits that survived a year or more had a growth of epithelium in the lungs. The only constant accompaniment of this epithelial proliferation was chronic inflammation with extensive formation of granulation and scar tissue either about an area of necrosis or in the wall of an ulcerated bronchus or bronchiole. These facts render it doubtful whether the tar was primarily concerned in the proliferation and metaplasia of the bronchial and alveolar epithelium.

Meyer,[21] from a study of experimentally produced epithelial proliferation following subcutaneous injection of scarlet red into the ear in rabbits, concluded that this occurs only with mild chronic inflammation and the growth of connective tissue associated with circulatory disturbances. We are inclined to accept this explanation of the changes observed in the lungs of our rabbits. We observed no proliferation of epithelium in any lung that was not associated with chronic inflammation and formation of granulation tissue. The epithelial growth was most marked in the lungs which showed the most extensive chronic inflammatory changes and particularly about organizing areas of necrosis. This last observation is interesting in connection with the belief of Stoeber and Wacker [22] that "an excess of products of protein decomposition may be a factor in upsetting cellular equilibrium and in causing

20. Fischer, B.: Frankfurt. Ztschr. f. Path. **27**:98, 1922.

21. Meyer, A. W.: Beitr. z. path. Anat. u. z. allg. Path. **46**:436, 1909.

22. Stoeber, H., and Wacker, L.: München. med. Wchnschr. **57**:947, 1900.

the onset of malignant proliferation." We are more inclined to believe that if the products of protein decomposition formed in the areas of necrosis had a part in inducing the epithelial proliferation observed in our animals their influence did not extend beyond the production of inflammatory hyperplasia.

Garschin [23] concluded from his experiments with *Kieselgur* (infusorial earth) and with cholesterol and tar that epithelium which rests on granulation tissue undergoes atypical proliferation and tends to grow downward into the underlying tissue but that when epithelium rests on fibrous tissue this type of change does not occur. He expressed the belief that the stimulus to growth lies not in the chemical nature of the substance employed to induce it but in the formation of granulation tissue immediately beneath the epithelium. This atypical epithelial proliferation is, in his opinion, part of a defense mechanism, because the growing epithelial cells tend to surround or cover the granulomatous tissue.[24] In the lungs of our rabbits receiving injections of the tar-oil mixture, the atypical character of the proliferating epithelial cells assumed two distinct forms: (1) masses of metaplastic, spindle-shaped or squamous cells (figs. 1, 2 and 3), and (2) numerous glandlike structures in atypical locations, lined with one or more layers of cells (figs. 1, 2, 4 and 5). Both types of change were definitely and constantly associated with granulation tissue. In the animals in which the tar-oil emboli did not induce necrosis followed by infection and organization, no epithelial proliferation occurred.

Furthermore, similar changes have been observed by other investigators, occurring either spontaneously or after the introduction into the lungs by various routes of numerous substances other than tar. Sternberg [25] described and illustrated similar epithelial proliferation about bronchi and abscesses in the lungs of guinea-pigs. Intratracheal insufflation of 5 cc. of a 0.25 per cent solution of hydrochloric acid by Winternitz, Smith and McNamara [26] induced epithelial changes in the lungs of rabbits in ten days that might have been "easily confused with a malignant neoplasm." Fischer [20] observed changes in the lungs of rabbits similar to those seen in our animals after intravenous injections of various substances dissolved in olive oil (e. g., camphor, oil of clove and creosote) and especially after injections of a neutral, incompletely purified liquid petrolatum.[27] Young [28] reported hyper-

23. Garschin, W. G.: Ztschr. f. Krebsforsch. **24**:492, 1927; **27**:481, 1928.
24. Garschin, W. G.: Ztschr. f. Krebsforsch. **27**:569, 1928.
25. Sternberg: Verhandl. d. deutsch. path. Gesellsch. **6**:134, 1904.
26. Winternitz, M. C.; Smith, G. H., and McNamara, F. P.: J. Exper. Med. **32**:205, 1920.
27. Gehes Codex, ed. 5, Dresden, Schwarzeck-Verlag G. M. B. H., 1929, p. 545.
28. Young, J. S.: J. Path. & Bact. **31**:265, 1928.

plasia and metaplasia in the serosa and glandlike epithelial changes in the superficial alveoli in the lungs of rabbits after intrapleural injection of sudan III and sodium cholate dissolved in olive oil. Willis and Brutsaert [29] exposed guinea-pigs to stone dust (silica) three or four times a week for from eighteen to thirty months. In the lungs of somewhat less than 10 per cent of these animals they observed "adenoma-like growths" originating from bronchial epithelium. "Connective tissue proliferation in these lungs was relatively slight." In all the experiments except those described in the last report cited, granulation tissue or chronic inflammation was present with the epithelial proliferation.

Proliferation of the alveolar and bronchiolar epithelium and alteration in its character are frequently observed about old apical tuberculous scars, in areas of fibroid phthisis and about organizing infarcts in the lung in man. In two recent autopsies one of us (J. P. S.) observed epithelial proliferation of moderate grade in the fibrous tissue about organizing infarcts in the lung. Except in primary carcinoma of the lung we have never observed, nor have we seen described or illustrated, such an extensive neoplasm-like growth of bronchial and alveolar epithelium in the human lung as was present in the lungs of many of our rabbits. It is probable, therefore, that rabbits possess a species peculiarity whereby the bronchial and alveolar epithelium may undergo extreme grades of hyperplasia and metaplasia without becoming neoplastic.

We are unwilling to state unreservedly that in any of our animals carcinoma actually developed, not only because the changes observed by us did not fulfil the criteria of malignant change specified by Murray and Woglom,[30] some of which it was impossible to apply in our animals. but chiefly because of the absence of discoverable metastases, the multifocal character of the growth, the intimate and constant association of the epithelial proliferation with granulation tissue and the occurrence of luxuriant epithelial growth in three weeks or less after the first injection of tar. But the epithelial growth in several of our rabbits was massive, locally invasive and atypical.

The character of the nuclei was not sufficiently determinative in any case to justify a positive diagnosis of carcinoma. There was a moderate variation in size but not to the extent commonly seen in malignant tumors. Most of the nuclei were vesicular. Mitoses were present in moderate numbers only. No atypical mitoses, such as triasters or

29. Willis, H. S., and Brutsaert, P.: Am. Rev. Tuberc. **17**:268, 1928.

30. Murray, J. A., and Woglom, W. H.: Experimental Tar Cancer in Mice, Scient. Rep. Invest. Imp. Cancer Research Fund **7**:45, 1921.

unequal division of the chromatin, were observed. Many cells contained nucleoli, but they were not large enough to serve as a criterion of neoplastic anaplasia as emphasized by MacCarty and Haumeder.[31]

In all cases the changes were of multiple origin, occurring wherever granulation tissue was formed. It is remarkable that such extensive and even atypical epithelial proliferation may take place, at least in the lungs of rabbits, without acquiring a definitely malignant character. The fundamental difference between massive inflammatory hyperplasia and neoplasm is still a mystery.

The lungs of many of our rabbits were certainly subjected to chronic irritation, first by the tar, then by the presence of necrotic tissue and finally by the superimposed chronic infection. In addition, there was interference with oxygenation and nutrition resulting from the vascular changes (thrombosis and endarteritis obliterans) and from the accumulation of exudate. Chronic irritation is generally accepted as an important etiologic factor in the development of carcinoma. But the mechanism by which it induces this result is not understood. It is conceivable that a chronic irritant may affect cellular proliferation in two ways: It may act as a direct stimulant to cell division, or it may act indirectly through the medium of the mechanical effects of the presence of necrotic tissue or the chemical effects of the products of disintegration of dead cells. In either case, successive attempts at repair by regeneration are continually thwarted by the persistent action of the irritant. It may be, therefore, that it is not merely the extent or the rate of cell proliferation but the constant frustration of normal repair that finally induces changes in the cells that render them malignant. It is evident from the degree of hyperplasia observed in our experiments that normal cells, at least those in the lungs of rabbits, possess a large measure of inertia that resists displacement across the ill defined line that separates inflammatory hyperplasia from true neoplasm.

SUMMARY

Repeated intravenous injections into rabbits of a mixture of liquid petrolatum and tar known to possess carcinogenic properties induced the following significant pathologic changes in the lung: tar-oil embolism, thrombosis, endarteritis obliterans, infarction with secondary infection, production of abundant granulation and scar tissue, formation of large cystlike cavities lined with epithelium and marked proliferation of bronchial and alveolar epithelium. The first three of these changes probably occurred in all the animals; the rest were observed in varying degrees in somewhat more than 50 per cent.

31. MacCarty, W. C., and Haumeder, E.: Am. J. Cancer **20**:403, 1934.

The epithelial proliferation was sufficiently massive and atypical in many animals to suggest strongly a malignant growth.

We are not convinced, however, that carcinoma was induced in any of our rabbits because of (1) the absence of discoverable metastases; (2) the multifocal character of the growth and (3) the intimate and constant association of the epithelial growth with granulation tissue without a definite relation to the amount of tar injected or the length of survival of the animal.

These experiments indicate the magnitude which inflammatory hyperplasia may attain, at least in the rabbit, without actually acquiring a malignant character.

ETIOLOGY OF BENIGN TUMORS

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Every type of tissue, even nerve cells,[1] is capable of some degree of repair. Repair is effected by division and differentiation of the undifferentiated cells of the part.[2] Besides pathologic stimuli, such as injury, there are physiologic stimuli to cell proliferation, e. g., the renewal of worn-out, effete cells, the proliferation of the tissue of the breast and endometrium during the sexual cycle and the response of the thyroid gland to various stimuli. When these stimuli become irregular or excessive, a pathologic condition develops. The results of such lesions, when progressive, may broadly be classed as (*a*) general hyperplasia (sometimes as hypertrophy) and (*b*) local hyperplasia or, if not regressive, benign tumor. Consideration of malignant tumors is outside the scope of this study.

HYPOTHESIS

A benign tumor may arise as the result of excessive or irregular physiologic stimuli or of local interference with the normal expression of such stimuli.

Benign uterine tumors afford the most obvious application of this hypothesis. Frank [3] and Frank and Rosenbloom [4] produced hyperplasia of the uterine musculature and endometrium in castrated rabbits and guinea-pigs with lipoid extracts of placenta and corpus luteum. Allen and Allen and Doisy [5] were able to produce hyperplasia of the uterus resembling that of puberty in immature rats with injections of an estrogenic substance. In women the uterus increases in size before each menstrual period, and an abrupt rise in the amount of the estrogenic substance in the blood is found seven days before the onset of the menses.

From the Department of Pathology, Woman's Medical College of Pennsylvania.

1. Hammett, F. S.: Protoplasma, **19**:117, 1933. Needham, J.: Chemical Embryology, London, Cambridge University Press, 1931.

2. Fried, B. M.: Medicine **10**:373, 1931. Reimann, S. P.: Am. J. Cancer **15**:2149, 1931.

3. Frank, R. T.: The Female Sex Hormone, Springfield, Ill., Charles C. Thomas, Publisher, 1929, p. 67.

4. Frank, R. T., and Rosenbloom, J.: Surg., Gynec. & Obst. **21**:696, 1915.

5. Allen, Edgar: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1932, p. 448. Allen, Edgar, and Doisy, E. A.: Am. J. Physiol. **69**:577, 1924.

It may therefore be taken as certain that this estrogenic substance causes hyperplasia of uterine muscle. Accumulating evidence suggests that this substance is also the cause of uterine myoma.

The arguments advanced in favor of excessive secretion of the estrogenic substance as the cause of uterine myoma have been set forth by Witherspoon [6] and are to be found in textbooks on gynecological pathology.[7] They will, therefore, not be recapitulated here.

Why generalized hyperplasia of the uterus should occur in some cases of excessive secretion of the estrogenic substance and myoma in others has not been explained. However, by analogy with what is known to take place in the thyroid gland and breast, it seems possible that in pure hyperplasia there is a continuous stimulation by the estrogenic substance and that in hyperplasia with myoma, the stimulation is intermittent, i. e., there are exacerbations and remissions of the process, the remission being followed by partial involution.

The origin of mucous polyp of the uterus is fairly easy to trace. In examining material from curettage of the uterus, especially in cases of hyperplasia, it is common to find groups of glands and sometimes also patches of stroma which differ in their appearance from the surrounding endometrium. In other words, these areas have not responded to the stimuli of the sexual cycle in the same way as the rest of the endometrium. These patches may be compared to the early stage of fibro-adenoma in the breast.[8] If such an area is not shed during menstruation, the glands and stroma are likely to proliferate during the next cycle. The process may be repeated in subsequent cycles, and thus a tumor is formed. If in a series of cases a uterine polyp is compared with the endometrium from the same patient it will be seen that cyclic changes similar to those in the endometrium can be traced in the polyp (figs. 1 to 6). Of course, the changes in the polyp are irregular and incomplete; otherwise, the polyp would be shed with the rest of the endometrium. Allowance also has to be made for the fact that the polyp is usually inflamed and the epithelial cells become filled with mucus, which somewhat alters the microscopic picture. But the influence of the sexual cycle, in the smaller polyps at any rate, can be easily made out.

If the reasons already enumerated point to an excess of the estrogenic substance as the cause of hyperplasia of the uterus, myoma and uterine polyp, the same can also be said of adenomyoma or endometriosis of the uterus. An examination of sections from a hyperplastic uterus may show that the glands penetrate much more deeply into the mus-

6. Witherspoon, J. T.: Endocrinology **17**:703, 1933.

7. Frank, R. T.: Gynecological and Obstetrical Pathology, ed. 2, New York, D. Appleton & Company, 1931, p. 214.

8. Ingleby, H.: Arch. Path. **14**:21, 1932; Lancet **2**:835, 1932.

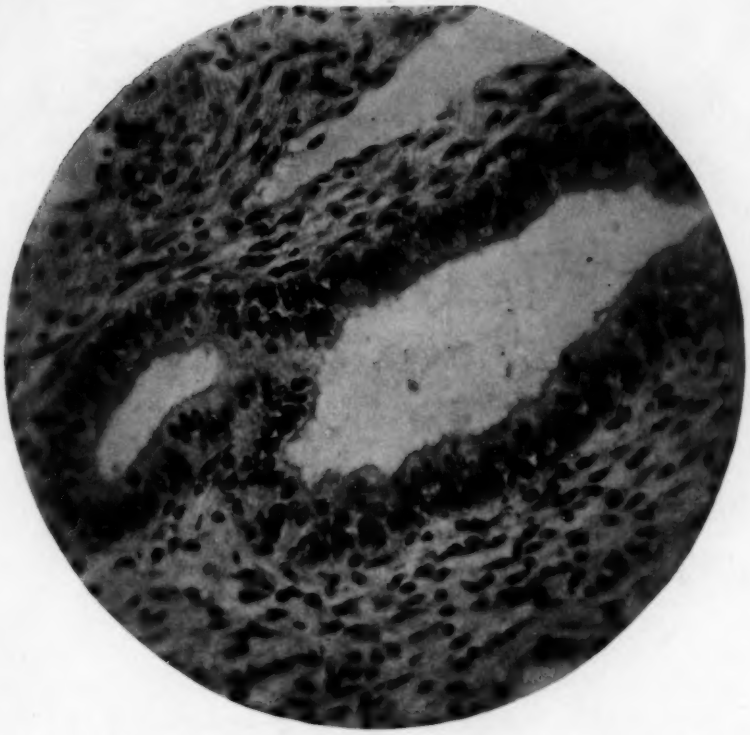

Fig. 1.—Mucous polyp of the uterus from a woman aged 48, removed fourteen days after a menstrual period. The glands show columnar epithelium with pseudostratification.

Fig. 2.—Endometrium from the patient from whom was taken the polyp shown in figure 1, presenting glands in a similar stage of development, i. e., with tall columnar epithelium and pseudostratification. One or two mitoses are visible.

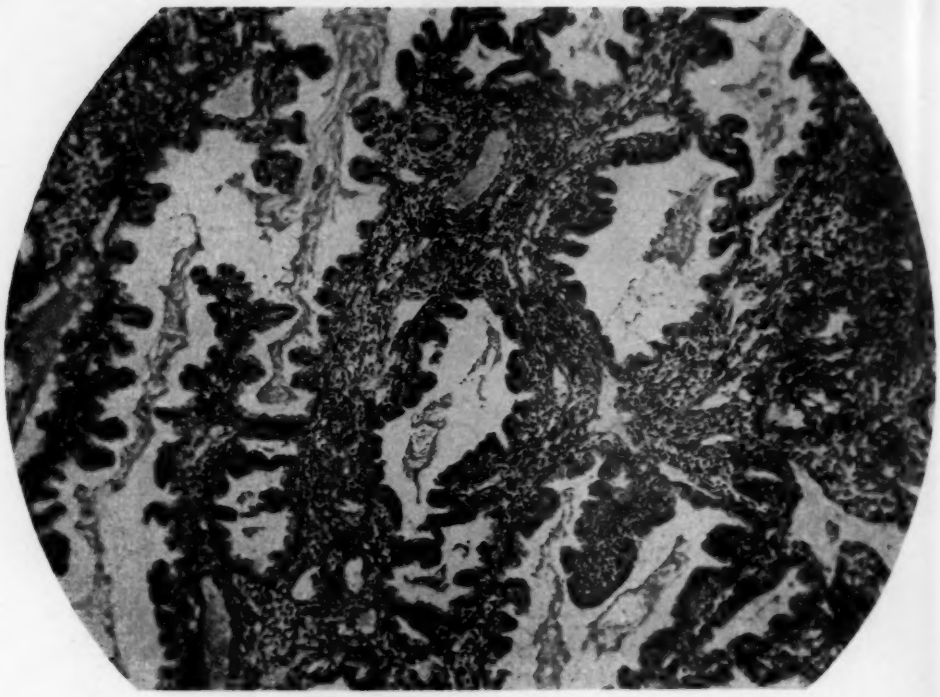

Fig. 3.—Uterine polyp removed from a woman aged 44 twenty-five days after a menstrual period. The glands are numerous and dilated and contain mucus. The epithelium is tufted.

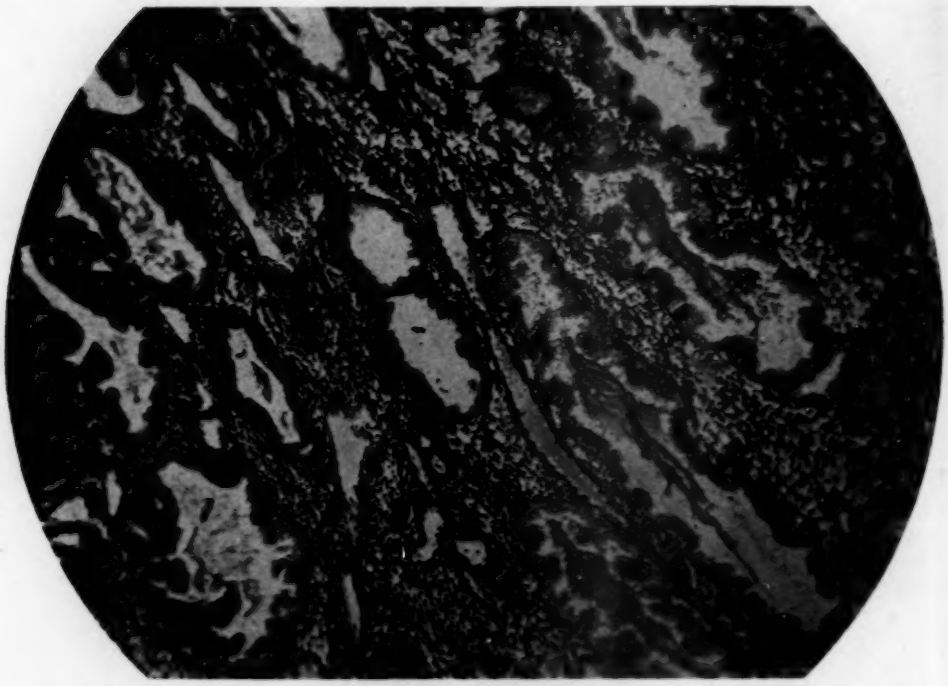

Fig. 4.—Endometrium from the patient from whom was taken the polyp shown in figure 3, presenting a rather irregular, premenstrual type of glands.

cularis than in a normal uterus. This may be due to hyperplasia on the part of the glands, but hyperplasia of the stratum submucosum of the uterine muscle is also and chiefly responsible. Processes of muscle are seen cutting off the uterine glands, and intermediate stages in the formation of well defined, diffuse adenomyoma are easily traced. In some of my cases, this process was associated with a peculiar form of cyst of the stratum granulosum in the ovary, but I am not yet in a position to state that this association is constant. A circumscribed

Fig. 5.—Uterine polyp from a woman aged 25, who had been menstruating for five weeks. The glands show pseudostratification, columnar epithelium and occasional mitoses. Part of a cystic gland appears in the picture.

hyperplasia of the muscle of the submucosa might, on the same hypothesis, give rise to the rarer circumscribed adenomyoma.

The pathogenesis of fibro-adenoma of the breast has been considered in detail elsewhere.[8] The conclusions reached have recently received interesting confirmation from a study of the correlation of the roentgenologic with the histologic appearance by Reimann and Seabold.[9] A

9. Reimann, S. P., and Seabold, P. S.: Am. J. Cancer **17**:34, 1933.

comparison of sections of breast and tumor from a series of cases in which the menstrual date was known showed that in each the cycle of changes in the breast was reflected in the tumor. During the premenstrual phase new lobules formed in the tumor as well as in the breast; during menstruation the epithelial cells of the tumor were also shed; in the postmenstrual phase involution of parts of the tumor was evident; in the interval phase the periductal tissue was dense and the epithelium was typical of the resting state. Of course, the tumor did

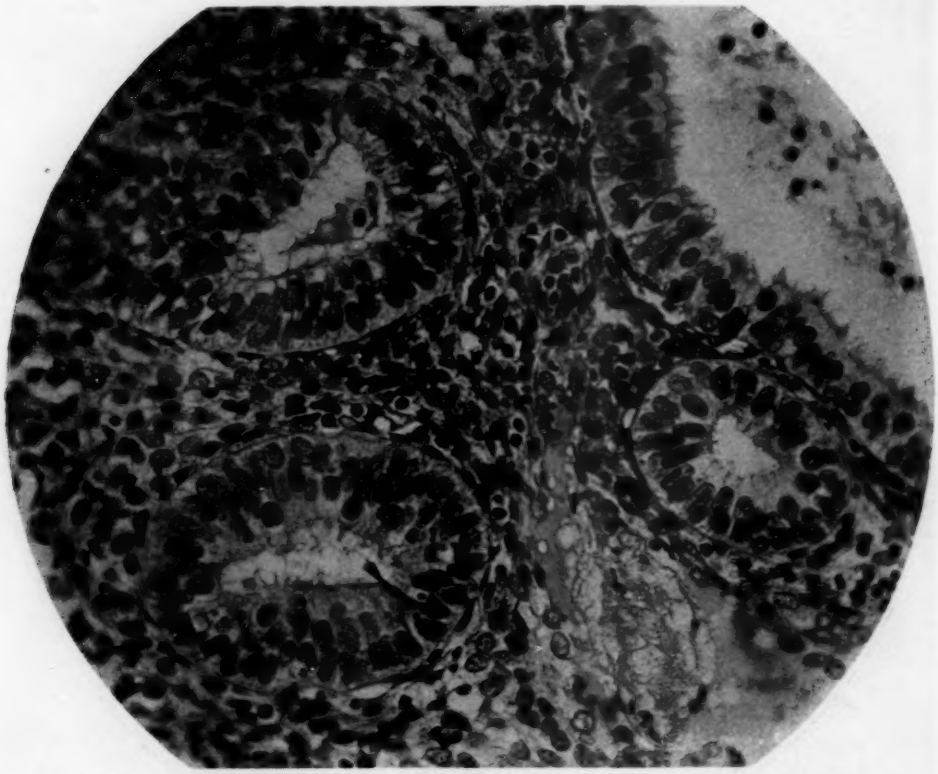

Fig. 6.—Endometrium from the patient from whom was taken the polyp shown in figure 5. The epithelium presents the same characteristics as the polyp. The edge of a cystic gland appears in the picture. The whole section showed well marked hyperplasia of the type due to excessive estrogenic substance ("swiss cheese" type).

not reflect the cycle perfectly. Had the growth and involution been perfect, there would have been no tumor. According to the hypothesis, it was precisely because there was interference with the proper response to physiologic stimuli that the tumor occurred. Probably, as Cheatle [10]

10. Cheatle, G. L., and Cutler, M.: Tumours of the Breast, Philadelphia, J. B. Lippincott Company, 1931.

has long maintained, fibro-adenoma has its origin in a general irregularity of the cycle, such as often occurs about the time of puberty. Figure 7 illustrates this type. When the cycle becomes regular, such a breast will become normal. But it may happen that in certain areas epithelial proliferation or fibrosis has become extreme, and a return to the normal condition is impossible. Permanent distortion of this kind results in fibro-adenoma.

The question of the origin of papilloma of the breast is complicated, and I do not propose to enter into it fully here. Certain types of intraductal proliferation seem to be definitely due to disturbance in hor-

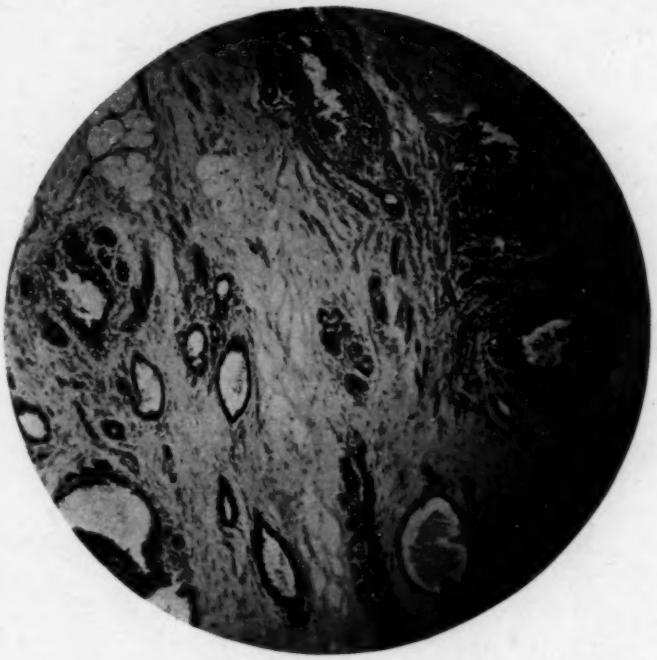

Fig. 7.—Tissue from the breast of a girl aged 17, showing irregular proliferation. A small fibro-adenoma was removed from the right breast. The menstrual periods, which began when she was 17 years old, were irregular, occurring about every two months. Menstruation took place forty-six days before operation, and not again until forty-six days afterward.

mones. Experimentally, it is possible to produce hyperplasia of the breast, cysts and intracystic proliferation in female rats by putting the animals with vasectomized males.[8] This renders the estrual cycle irregular (figs. 8 and 9). Other investigators have shown that endometrial changes also take place under these conditions.[11]

11. Hisaw, F. L., and Leonard, S. L.: Am. J. Physiol. **92**:574, 1930. Long, J. A., and Evans, H. M., in Memoirs University of California, Berkeley, Calif., University of California Press, 1922, vol. 6, p. 1.

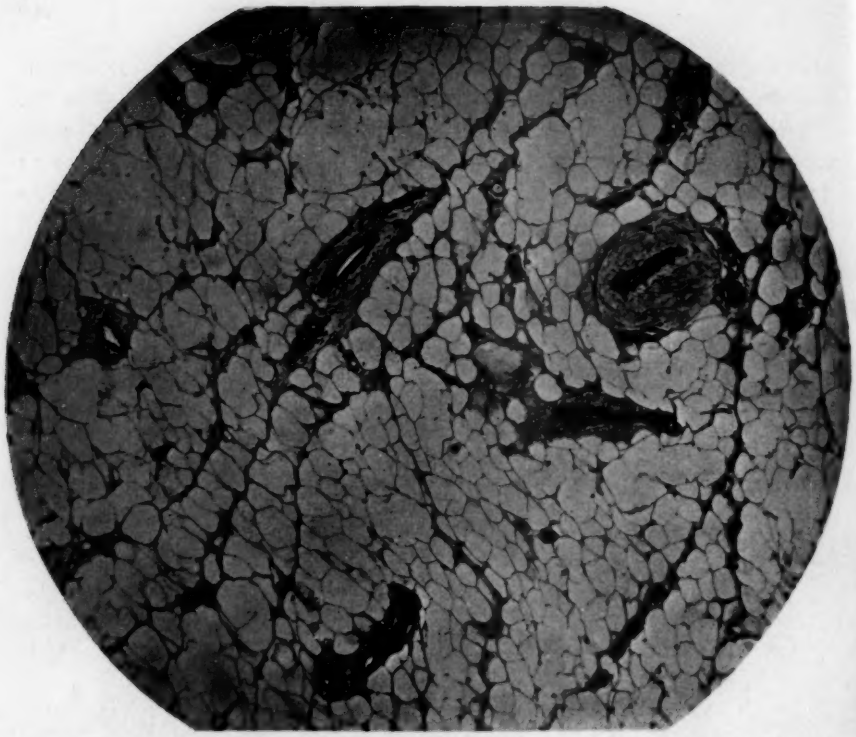

Fig. 8.—Normal tissue from the breast of a rat to be compared with that in figure 9.

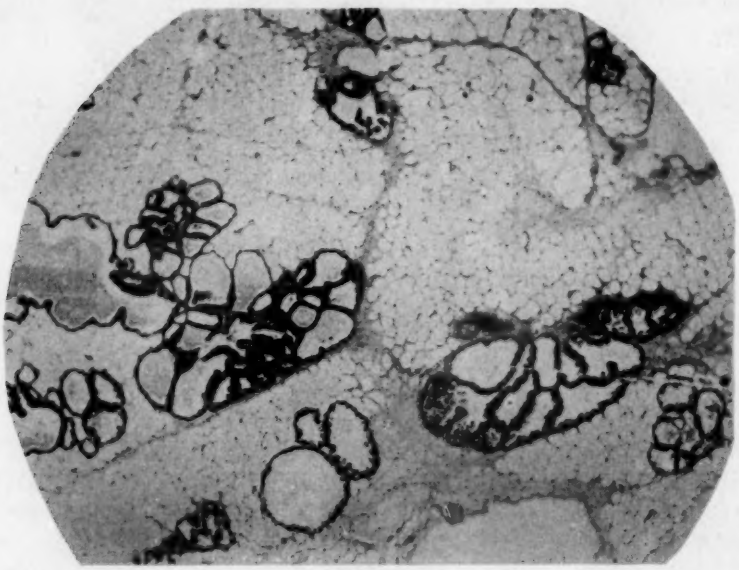

Fig. 9.—Tissue from the breast of a rat showing changes induced by rendering the estrual cycle irregular.

Rather seldom is there a chance to examine tissue from the endometrium and breast of the same patient, except post mortem, when the endometrium is generally in a state of poor preservation. Three patients came under my observation in whom this was possible. In one, a single woman of 35, the endometrium showed well marked hyperplasia of the type due to excessive estrogenic substance ("swiss cheese"), as shown in figure 10. Eighteen months later a cyst was removed from

Fig. 10.—Endometrium from a woman of 35, showing well marked hyperplasia of the type due to excessive estrogenic substance ("swiss cheese" type).

the breast. During this time the periods had been irregular. The sections showed desquamative epithelial hyperplasia with intracystic proliferation comparable to the artificially produced lesions in the rat (fig. 11). The second patient, a woman of 48, underwent curettage and the removal of a cyst from the breast at the same operation about three weeks after the menstrual period. Hyperplasia of the endometrium of the type due to secretion of estrogenic substance and corpus

luteum occurred,[12] and the breast showed a cystic condition. One cystic area was compressing the surrounding tissue and becoming encapsulated to form a fibro-adenoma. The rest of the tissue showed moderate epithelial proliferation. The third patient, a married woman of 38, had hyperplasia of the uterus and endometrium, the latter being of the type due to estrogenic substance (the "swiss cheese" type). Multiple cysts of the left breast also occurred, which showed desquamative

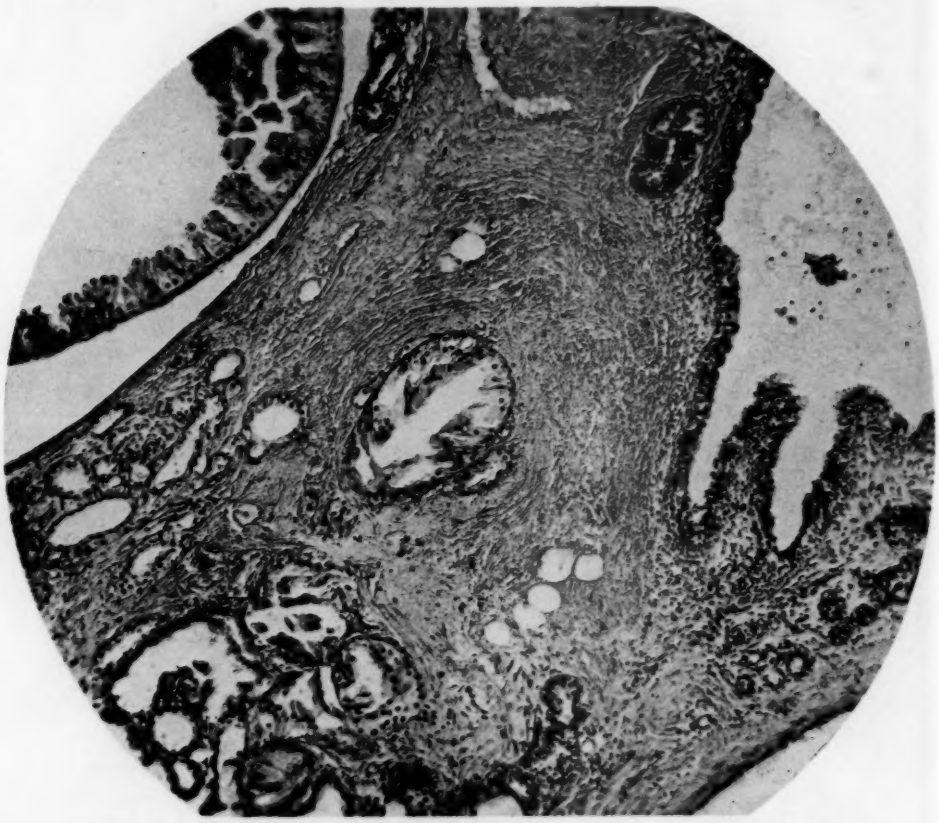

Fig. 11.—Tissue from the breast of the patient from whom was taken the endometrium shown in figure 10, presenting desquamative epithelial hyperplasia.

epithelial hyperplasia and slight intracystic proliferation. The lesions in both the breast and the uterus resembled those of the first patient. Further observations are needed, but enough has been said to show that some forms of epithelial hyperplasia in the breast are linked with alteration of physiologic stimuli.

12. Burch, J. C.; Williams, W. L., and Cunningham, R. S.: Surg., Gynec. & Obst. **43**:338, 1931. Ingleby, H.: Bull. Internat. A. M. Museums **13**:125, 1934.

The best recognized example of the power of excessive stimulation to produce a benign tumor is in the thyroid gland. Rienhoff [13] has shown clearly that in fulminating cases of exophthalmic goiter there is a generalized hyperplasia of the thyroid and that the thyroid gland in patients with exacerbations and remissions is characterized by nodular changes. In nodular or adenomatous goiter, there seems to be no doubt that the so-called adenoma represents areas of excessive hyperplasia, arising in response to a demand by the body for more thyroid secretion, which subsequently have not undergone involution. "Fetal adenoma" has a similar origin.[14] Whether the same can be said of the type of adenoma of the thyroid gland which consists of branching columns of cells is more doubtful. Ewing [15] classed this with fetal adenoma. Boyd [16] considered it a separate type of tumor, to which he gave the name "true adenoma." However, similar appearances are noted in the developing thyroid gland of the new-born infant, so that it seems justifiable to consider it a type of "fetal adenoma."

The benign tumor found in the cortex of the suprarenal seems comparable to adenoma of the thyroid gland. During various periods of life as well as during the menstrual cycle the suprarenal cortex is undergoing growth and regression. Local hyperplasia is common, especially in persons with high blood pressure, and this may enlarge to the proportions of an "adenoma."

The production of tumor by excessive repair is well known and has been studied experimentally by Hammett and Smith [17] and by Reimann [18] in the regenerating claws of hermit-crabs, using sulphydryl as the stimulus to cell division. Tumor growth arose secondarily in claws in response to a break in the confining envelop of chitin. The removal of mechanical restraint caused more cells to be produced than were needed for simple repair, and the intrusion of a new set of ratios needed for repair into the normal ratios already active for regeneration shifted the normal time relations of proliferation, differentiation and organization. Reimann expressed the belief that overrepair in cases of keloid is due to shifts in these time relations. Of course, as the authors have been at pains to point out, all tumors are the result of faults of proliferation, differentiation and organization. When these processes are understood, the cause of tumors will be known.

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13. Rienhoff, W. F.: Bull. Johns Hopkins Hosp. **37**:285, 1925.

14. Graham, Allen: Am. J. Surg. **7**:163, 1929.

15. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1928, p. 946.

16. Boyd, W.: Pathology of Internal Diseases, Philadelphia, Lea & Febiger, 1931, p. 513.

17. Hammett, F. S., and Smith, D. W.: Protoplasma **13**:18, 1931.

18. Reimann, S. P.: Arch. Path. **15**:675, 1933.

Another example of a tumor apparently brought about by overrepair is the pigmented cutaneous nevus. Masson [19] traced the origin of this tumor, which behaves exactly as a cut nerve behaves when attempting to regenerate. He proved a similar origin for carcinoid of the appendix, the so-called argentaffin cell tumor. An exaggerated response to inflammation seems to be the cause of most papillomas of the skin and mucous membranes, but, as in other pathologic conditions, similar results may be brought about by different agents, and it is not possible to assign one cause to the production of all types of papilloma.

The study of a small papillary adenoma of the kidney, found recently in a routine autopsy, seems to throw light on the etiology of these tumors. The tumor lay just under the capsule and was barely visible to the naked eye. There was general dilatation of all the convoluted tubules lying near the surface. The dilated tubules occupied roughly the outer fourth of the cortical zone. No cause could be found for the dilatation. It was not present in other parts of the kidney. Under a low power lens the tumor stood out distinctly from the surrounding tissue and had slightly compressed the adjacent tubules. It had no fibrous capsule, however, and, under a high power lens the renal tubules and the tissue at the edge of the tumor were so much alike that if only a small field had been examined, a tumor would scarcely have been suspected. The entire tumor consisted in the main of slender cores of fibrous tissue covered by epithelial cells resembling those of the convoluted tubules. These papillae projected into a cystic space. But an examination of the edge of the tumor revealed clearly that the papillae were derived from dilated renal tubules. The dilatation had become so extreme that the walls of the tubules had given way. What remained was the interstitial tissue which originally lay between two tubules and now formed the core of a papilla and the epithelial cells which once lined the tubules and now covered the papilla.

Of course, there is no reason why epithelium and connective tissue should not continue to proliferate in their new orientation. They undoubtedly do in most instances. As a cyst enlarges, the lining epithelium must proliferate sufficiently to keep pace with the increased area to be covered, unless circumstances, such as excessive intracystic pressure, make proliferation impossible. Since it is the nature of epithelium to grow over a "raw" surface, this behavior may be thought of as physiologic. Nevertheless, it is common to see epithelial proliferation outstripping physiologic limits. In an abnormal environment it may be expected to do so. A point of interest which is clearly illustrated in this tumor is the alteration in polarity of the epithelial cells. In the tubules outside the tumor, the nuclei are basal. In the dilated

19. Masson, P.: Ann. d'anat. path. **3**:27, 1926.

tubules of the tumor itself, the nuclei have begun to shift toward the center of the cell, but many cells still retain their brush border. In the middle of the tumor, where all tubular structure is lost, polarity is likewise lost, and a brush border is rarely seen. Probably the loss of polarity is the result of loss of function.

The papillary adenoma of the kidney already described illustrates the formation of a benign tumor in association with excessive and abnormal secretion into a closed space. This mechanism may help to explain the origin of tumors in other organs also. Undoubtedly, it plays a part in the development of some papillary tumors of the breast.

At first it seemed that the teratoma was outside the scope of the hypothesis under consideration. It could, of course, be considered an example of distorted physiologic growth, but why totipotent cells should proliferate in any situation is obscure. However, Hammett and D. W. Smith [17] and Reimann [18] recently succeeded in producing teratoma in a certain proportion of regenerating claws of crabs by the use of sulphydryl. They found that sulphydryl, the normal stimulus to cell division, was capable of stimulating proliferation to the extent of the production of aberrant, unorganized growths.

It is not my aim to discuss the pathogenesis of every benign tumor. In regard to many of them, it is idle to speculate until the facts which would throw light on their origin are forthcoming. A working hypothesis is put forward in the hope of furnishing a clue to the direction in which to seek, for an understanding of the cause of benign growths is the first step toward their prevention.

It was not until after this paper had been written that my attention was called to the lectures of G. W. Nicholson,[20] in 1925, on "The Nature of Tumor Formation." He stated: "The course development takes in every tissue and the form its cells ultimately assume are not fixed by law, natural or otherwise, but depend mainly upon the conditions to which the cells are subjected during the whole of their life history." To this opinion, I cordially subscribe.

SUMMARY

The pathogenesis of certain benign tumors is explained on the hypothesis that benign tumors arise as the result of excessive or irregular physiologic stimuli or of local interference with the normal expression of such stimuli. More facts are needed, but it appears probable that the hypothesis will be found to hold for all types of benign tumor.

20. Nicholson, G. W.: The Nature of Tumor Formation, Cambridge, W. Heffer & Sons, 1926, p. 42.

JUVENILE DEMENTIA PARALYTICA

XII. GROSS AND MICROSCOPIC PATHOLOGY

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The pathologic changes observed in cases of juvenile dementia paralytica are in many points distinctly different from those present in the acquired form of the disease. Because of the comparative rarity of the disease far fewer studies have been made, and there are yet some controversial points regarding the nature of the pathologic process. This article is devoted to the pathologic changes observed in the nervous system and is one of a series of studies on juvenile dementia paralytica.[1] The entire series has been based on 43 cases personally studied and on reports of 610 cases published in the literature; 188 of the patients were examined post mortem.

Seven of my own cases came to postmortem examination. Dr. Walter Freeman of Washington, D. C., assisted in the study of these cases and furnished the photomicrographs.

There have been many special studies of the pathologic changes observed in juvenile dementia paralytica; such studies have been reported by Homén,[2] Westphal,[3] Alzheimer,[4] Dees,[5] Mott,[6] Watson,[7]

From the Menninger Clinic.

1. Menninger, W. C.: Juvenile Neurosyphilis with Dementia Paralytica: I. Incidence, Sex, and Age at Onset, Am. J. Syph. & Neurol., October 1934; II. Family History, Arch. Int. Med., to be published; III. Developmental History, J. Nerv. & Ment. Dis., to be published; IV. Cranial Nerve and Motor System Syndromes, Arch. Neurol. & Psychiat., to be published; V. Sensory Changes and the Reflexes, Ann. Int. Med., to be published; VI. Physical Complications, Stigmata, and Endocrinopathies, Am. J. Syph. & Neurol., to be published; VII. The Descriptive Mental Picture, Am. J. Psychiat., to be published; VIII. The Psychology of Juvenile Dementia Paralytica, Psychoanal. Rev., to be published; IX. Laboratory Findings, J. Lab. & Clin. Med., to be published; X. Clinical Course, Including Prodromal Symptoms, Nature of Onset, Remissions and Duration, Am. J. Syph. & Neurol., to be published; XI. Treatment, ibid., to be published.

2. Homén, E. A.: Neurol. Centralbl. **9**:514, 1890.

3. Westphal, A.: Charité-Ann. **18**:732, 1905.

4. Alzheimer: Allg. Ztschr. f. Psychiat. **52**:533, 1895.

5. Dees: Psychiat. Wchnschr. **1**:192, 1899.

6. Mott, F. W.: Arch. Neurol. Path. Lab. London County Asylums **1**:250, 1899.

7. Watson, G. A.: Arch. Neurol. Path. Lab. London County Asylums **2**:621, 1903.

Burzio,[8] Sträussler,[9] Trapet,[10] Rondoni,[11] Lafora,[12] Rezza and Vedrani,[13] Scharnke,[14] Bielschowsky,[15] Ricksher,[16] Springlová,[17] Juba [18] and Stewart.[19] Consequently the purpose of the present report is primarily to present the special points seen in my own series of cases, in addition to a summary of previous observations.

It is often stated that the pathologic changes in juvenile dementia paralytica are similar to those occurring in the adult form of the disease. The cortical changes are similar, although they are usually more extensive in the juvenile form, and the ependymitis granulosa is common to both forms. Beside these two points, several features are found more commonly and characteristically in the juvenile form: In the gross examination, atrophy, leptomeningitis and pachymeningitis, hydrocephalus, and more extensive ependymitis granulosa are observed; in the microscopic picture there is a tendency of the degenerative changes to exceed the inflammatory ones; the neuroglia and microglia show excessive proliferation, and binucleated Purkinje cells are numerous in the cerebellum.

GROSS PATHOLOGIC CHANGES IN THE BRAIN

Atrophy.—Atrophy is consistently observed, although most reports fail to make a distinction between hypoplasia and atrophy. In many instances the gross as well as the microscopic picture is definitely one of atrophy, and yet, as judged from the similarities seen in the brains of feebleminded children in which hypoplasia is frequent, it is certain that the small brain so often found may be due in part to hypoplasia. Hypoplasia was especially mentioned by Stewart,[19] and Southard and Solomon [20] called attention to the possibility that such a condition was present in a patient who had had a period of normal development. This feature is certainly to be expected in cases in which the child has been defective from birth, and it may occur in any case in which the spirochetosis is congenital.

8. Burzio, F.: Ann. di freniat. **15**:33, 1905.
9. Sträussler, E.: Jahrb. f. Psychiat. **27**:7, 1906.
10. Trapet, A.: Arch. f. Psychiat. **45**:716, 1909.
11. Rondoni, P.: Arch. f. Psychiat. **45**:1004, 1909.
12. Lafora, G. R.: Ztschr. f. d. ges. Neurol. u. Psychiat. **15**:281, 1913.
13. Rezza, A., and Vedrani, A.: Riv. ital. di neuropath. **6**:254, 1913.
14. Scharnke: Arch. f. Psychiat. **55**:302, 1914.
15. Bielschowsky, M.: J. f. Psychol. u. Neurol. **22**:84, 1916.
16. Ricksher, C.: Alienist & Neurologist **39**:8, 1918.
17. Springlová, M.: Časop. lék. česk. **64**:1833 and 1880, 1925.
18. Juba, A.: Ztschr. f. d. ges. Neurol. u. Psychiat. **141**:702, 1932.
19. Stewart, R. M.: J. Ment. Sc. **79**:602, 1933.
20. Southard, E. E., and Solomon, H. C.: Neurosyphilis, Boston, W. M. Leonard, 1917, p. 440.

The atrophy is usually most pronounced in the frontal region (Schmidt-Kraepelin,[21] Mott,[6] Ricksher[16]); occasionally it is present in the parietal region (Mott and Ricksher), and Alzheimer[4] reported having observed a softening of the temporal lobe in 1 case and shrinkage of the medulla and pons frequently. The convolutions are often of a simple type, with widening of the sulci. Mott[6] reported that in 6 cases when the two halves of the brain were weighed the left hemisphere weighed less than the right, the difference being an average of 28 Gm. in favor of the right hemisphere. A report of a similar observation was given by Watson.[7] Freeman[22] reported the weight of two brains to be 950 and 540 Gm. and called attention to the fact that the optic nerves are often atrophic and that sclerosis of the optic tectum is found in nearly all the cases of juvenile dementia paralytica.

In the following case atrophy of the frontal lobes and of the left optic nerve was observed.

CASE 39.—A boy, aged 12, was brought to the hospital because of convulsions. His past history was not obtainable.

The patient was well nourished but was much undersized, weighing only 59 pounds (26.8 Kg.). There were no gross abnormal findings. Neurologic examination showed Argyll Robertson pupils. The gait was ataxic and spastic, and the patient walked with the knees drawn together and the legs turned out, so that he stepped on the inner sides of his feet. There were tremor of the hands, hyperactive knee jerks but depressed ankle jerks, ankle clonus and a Babinski sign. The patient had moderate microcephaly, facial asymmetry, beveled incisors and a clouding of the left cornea. He showed a basic mental age of 3 years, was extremely childish in all his behavior and frequently exhibited masturbation. The laboratory examination showed positive Wassermann tests of the blood and spinal fluid; the fluid contained 79 cells per cubic millimeter and an increased amount of globulin.

The patient had from five to ten generalized convulsive seizures a month, and persistent treatment with mercury produced no result. The condition rapidly progressed to death.

Autopsy showed that the pia was thickened and adherent to the brain cortex, which showed congestion and formation of new vessels. There were marked sclerosis and shrinkage of the brain, particularly along the frontal bases. The left optic nerve was atrophied to one-half the usual size. The cerebrospinal fluid was greatly increased but the amount was not measured. The gyri recti were notably narrowed, so much so that the olfactory lobes hung in the central fissure. Microscopic examination showed extreme sclerosis of all the layers of the cerebral cortex. The blood vessels were congested, and the perivascular spaces were filled with plasma cells and lymphocytes. All the varieties of neuroglia cells were seen proliferating about the capillaries and about the neuron cells, which were numerically reduced. The Betz cells were elongated, diamond-shaped and extremely pigmented, and they showed a fragmentation of the chromatin granules. Many nuclei were vacuolated, and many showed absence of staining properties. Many

21. Schmidt-Kraepelin, T.: Ueber die juvenile Paralyse, Berlin, Julius Springer, 1920.

22. Freeman, W.: Neuropathology, Philadelphia, W. B. Saunders Company, 1933, p. 217.

new blood vessels were seen, particularly in the upper layer of the cortex, and the pia was heavily infiltrated with lymphocytes and plasma cells, bearing also an overgrowth of connective tissue.

Leptomeningitis and Pachymeningitis.—The dura is often normal (Watson [7]) but may be thickened (Schmidt-Kraepelin [21]), and Ricksher [16] stated that a hemorrhagic pachymeningitis is common. It did not occur in any of my cases. The pia, on the other hand, is always thickened and infiltrated (Watson [7]) and in some cases is reported to be as much as 4 mm. thick (Ricksher [16]). Trapet [10] was so impressed with the degree and frequency of the leptomeningitic process that he regarded the disease as a meningo-encephalitis. In the 7 cases in my series the pia was thickened, the thickening often being very marked.

Hydrocephalus.—According to Mott [6] and Watson,[7] the ventricles are invariably dilated, but Ricksher [16] stated that "sometimes a true hydrocephalus occurs," inferring that such an occurrence is unusual. Freeman [22] reported a case in which extreme hydrocephalus was observed and stated that in 2 patients examined he found, respectively, 360 cc. and 710 cc. of spinal fluid. In all my patients there was some dilatation of the ventricles.

Ependymitis Granulosa.—As in adult dementia paralytica, the granular character of the fourth ventricle is consistently found. In addition, the lining of the lateral ventricle is often the site of a proliferation of fibrillar neuroglia, with the characteristic roughened surface.

The following case showed a marked ependymitis granulosa and a cystic choroid plexus.

CASE 40.—The patient was a colored woman, aged 24. The family history was not known. The patient had reached the fifth grade, but she had never done well in her school work. She was known to have been very careless and dirty in her habits all her life. She learned to use alcohol, snuff and chewing tobacco, all in excessive amounts, at about the age of puberty. It is presumed, though not definitely known, that she had had convulsive seizures for several years. The onset of the disease was vague and there was no definite change in her behavior until a month before her admission, when psychotic symptoms developed.

Physical examination showed no gross abnormalities. Neurologic examination showed a defect of speech, tremor of the tongue and extended fingers and irregularity and inequality of the pupils, the left pupil being fixed. The knee jerks were at first exaggerated but later disappeared. There was a facial paralysis on the right side. There were no stigmas. The patient was confused mentally and disoriented. She described visual and auditory hallucinations which centered around her paranoid delusion of persecution by unseen forces and people. She showed marked mental regression and a defective memory. The laboratory examination showed a positive Wassermann reaction of the blood and spinal fluid; the fluid contained 51 cells per cubic millimeter, and the amount of protein was increased.

The patient was given a mixed treatment for a time and improved slightly, sufficiently to be able to work in a laundry. She complained of attacks of vertigo and of headaches. Later delusions of grandeur and tactile hallucinations developed;

the patient became more confused and entirely apathetic, required feeding with a tube and died in a convulsive seizure six months after admission.

Autopsy showed that the pia was thickened and the brain markedly shrunken, sclerotic vessels being apparent. Both lateral ventricles were moderately dilated, and the ependyma, as well as the fourth ventricle, was roughened. The amount of cerebrospinal fluid was increased. The choroid plexuses were cystic, as observed macroscopically.

Microscopic examination showed a marked sclerosis of the cortex, with many lymphocytes and plasma cells about the blood vessels. There was a marked degenerative change in the area of the lenticular nucleus. There was an extensive proliferation of neuroglia tissue, with severe acute changes in the ganglion cells.

Cerebellum.—Sträussler [9] was of the opinion that juvenile dementia paralytica is primarily a developmental disturbance localized chiefly in the cerebellum. He believed that there are anatomic signs similar to those present in other hereditary diseases of the cerebellospinal system, notably Marie's cerebellar ataxia and, more distantly, the juvenile form of amaurotic idiocy. The cerebellum grossly shows atrophy of the folia, and on section extreme gliosis is apparent. Next to the smallness of the cerebral hemispheres, these cerebellar changes were regarded by Stewart [19] as the most important pathologic point of differentiation from adult dementia paralytica. Contrary to the majority of opinions, Watson [7] found that the cerebellum showed changes less marked than those observed in the cerebrum.

Formation of Gummas.—Freeman [22] stated that gummatous meningo-encephalitis is a frequent accompaniment of juvenile dementia paralytica. Schmidt-Kraepelin [21] reported one case of five years' duration in which in addition to the infiltrative process there was a gumma in the optic thalamus. A similar case with a gumma was also recorded by Brissot.[23] Grütter [24] reported two cases of juvenile dementia paralytica in a brother and a sister; the boy had a gumma of the cortex in the ventral surface of the left frontal lobe.

MICROSCOPIC OBSERVATIONS

The microscopic changes seen in juvenile dementia paralytica are of two general types: inflammatory, including productive changes, and degenerative. In cases of shorter duration the inflammatory changes may predominate, but in cases of long standing the degenerative changes are the more pronounced. In addition, some writers stress the developmental disturbances.

Inflammatory Changes.—The exudative and proliferative changes are usually associated. The exudative process is obvious throughout the brain, but particularly there is a marked infiltration with round cells about the blood vessels and meninges. The accumulation of

23. Brissot, M.: Ann. méd.-psychol. **72**:663, 1914.

24. Grütter, E.: Ztschr. f. d. ges. Neurol. u. Psychiat. **54**:225, 1920.

lymphocytes and plasma cells about the blood vessels is similar to that seen in the acquired form of dementia paralytica. The meningeal response is usually more marked in the juvenile form of the malady, so much so that Trapet [10] regarded the disease as a meningo-encephalitis. This is also due in part to the associated proliferative changes.

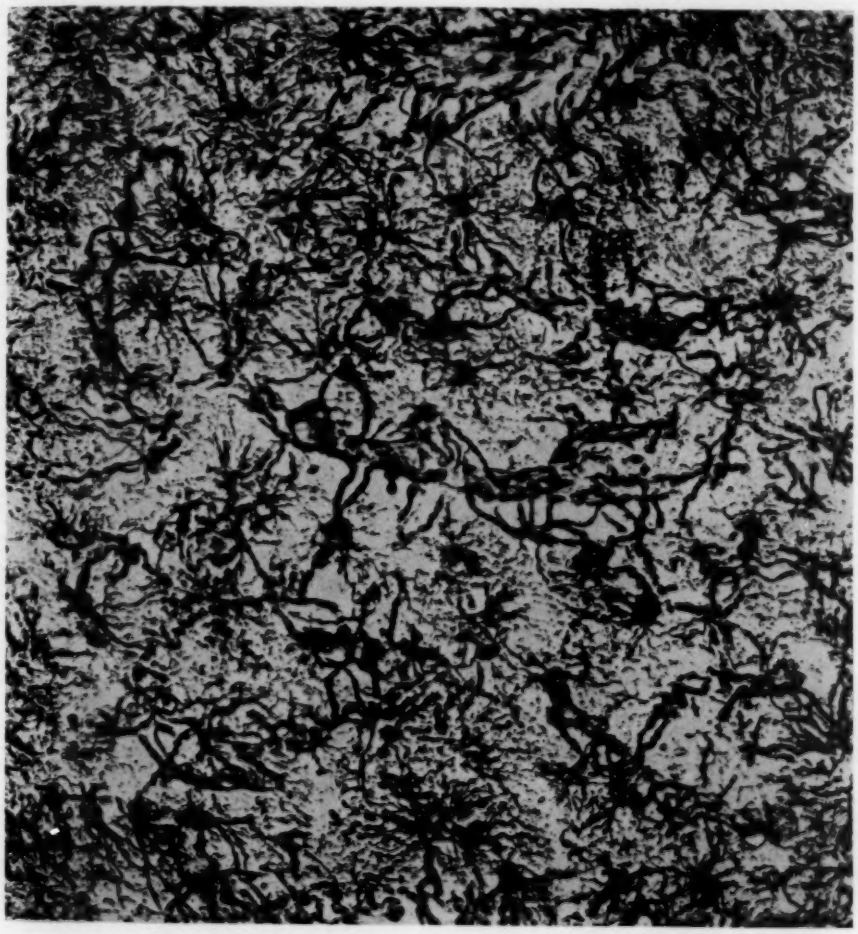

Fig. 1.—Photomicrograph of glia in dementia paralytica, showing the fibrillary form and hypertrophy (Cajal impregnation; × 600).

The proliferation of neuroglia and microglia is always marked, as stressed by Watson,[7] Ricksher,[16] Mott,[6] Freeman [22] and Stewart.[19] The result of this enormous proliferation is a marked change in the architectonics of the cortex; the density is much increased (fig. 1). Stewart stated that this proliferation is most marked beneath the pia

and in the periventricular region. The gliosis is often marked around the blood vessels. The roughened ependyma of the ventricles is the result of proliferating neuroglia, and often only a remnant of the ependymal layer remains. As in adult dementia paralytica, the microglia cells in the cortex hypertrophy to form the rod-shaped stab cells (fig. 2). Iron is present in the microglia as in the adult form of the

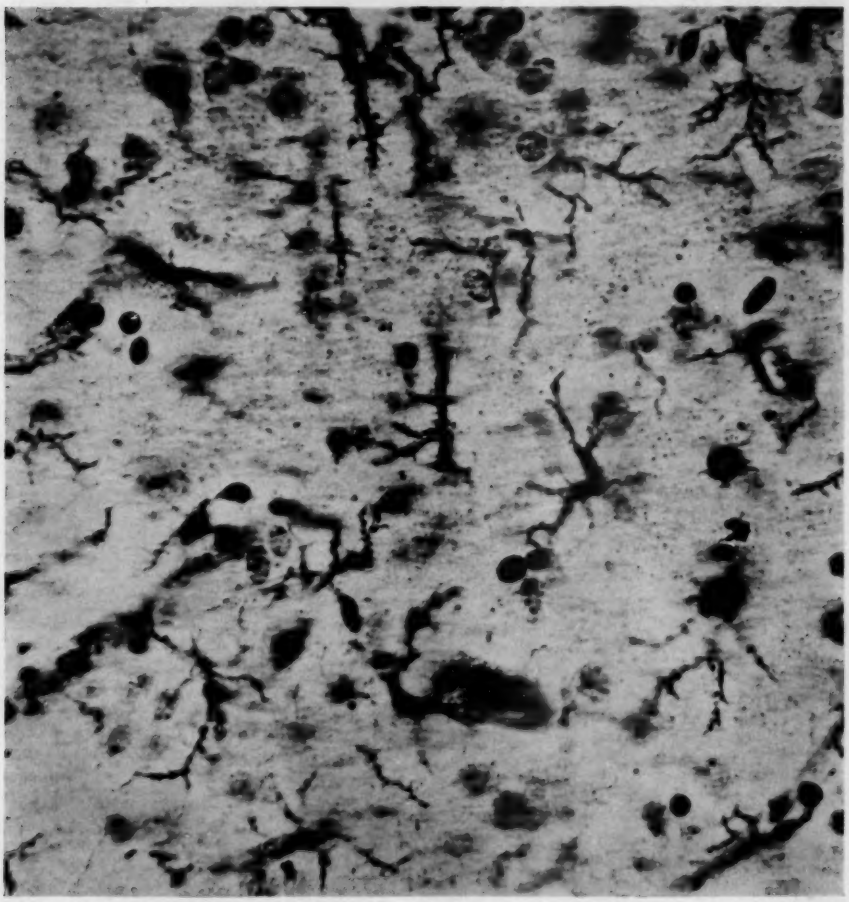

Fig. 2.—Photomicrograph showing hypertrophic microglia cells in dementia paralytica (Hortego impregnation).

disease, and many phagocytic glia cells engulf globules of fat. Iron and fat may be present in large quantities.

In the following case there was an extensive overgrowth of the neuroglia.

Case 41.—The patient was a boy, aged 12 years. The maternal grandfather was addicted to the use of alcohol, and two first cousins showed serologic evi-

dence of neurosyphilis. Dementia paralytica developed in the mother at the birth of the patient, and she gained a remission following malaria therapy. She had had six pregnancies; only three children were living. The patient was born at 7 months and was weak and emaciated. At the age of 9 months he was given a course of arsphenamine, and later he received injections of mercury. Because of a gonorrheal ophthalmia acquired at birth, the right eye had to be removed when the patient was 8 years old. He did fairly well physically and mentally until 3 years of age, when a disturbance in gait began to develop. He was sent to school when he was 5½ years old, but he failed to progress. He failed from that time on, and on admission he presented contractures, made no sign of recognition of his environment, had to be fed with a spoon and was entirely incontinent. He appeared to be about 6 years old; he lay in bed making gurgling sounds but not uttering words. The legs were flexed with contractures; there was marked emaciation, and bullous lesions of varying sizes were scattered over the body. The neurologic examination showed a retraction of the head and a contracture of the right arm. The right eye had been surgically removed; the left cornea was opaque, and the pupil did not react to light. The deep reflexes were exaggerated, and there was a sustained ankle clonus but no Babinski sign. The feet were in equinus varus position, and the legs could not be straightened. The patient was incontinent. A saddle-shaped nose and infantile genitalia were noted. Only about one half of the teeth were erupted; these were poorly developed, and some were notched. The mental status was as previously described. The laboratory examination showed a strongly positive Wassermann reaction of the blood and spinal fluid; the spinal fluid contained 34 cells per cubic millimeter; the amount of protein was much increased; the colloidal gold curve was 3334432000, and the mastic reaction, 455554.

Despite the extremely poor condition on admission, the patient lived for nearly a year. He was given a course of malaria therapy but without any apparent effect.

At autopsy the macroscopic examination showed that there was atrophy of the cerebrum; the convolutions were broad and the fissures shallow. The meninges were thickened, and the ependyma of the ventricles was very granular. The cerebellum was firm, with an atrophic cortex. The ventricles were dilated and the basilar structures atrophic; the optic nerves were likewise atrophic. The microscopic examination showed a marked thickening of the meninges, especially of the pia, and a round cell infiltration of lymphocytes and plasma cells. The cortex showed a marked increase in the vascular elements, with congestion and proliferation of all the coats of the vessels. There was a degeneration of the nerve cells and of many rod-shaped microglia cells. The mesencephalic region showed the most marked changes, with marked gliosis, infiltration of round cells, a marked increase in the vascular elements and a degeneration of nerve tissues. The cerebellum showed many binucleated and trinucleated Purkinje cells with the cell bodies apparently well preserved.

The changes in the blood vessels vary widely, but usually the vessels show more proliferative than degenerative changes; often the proliferative changes are very marked and involve all the layers of the vessel wall. According to Watson [7] the arteriocapillary lesions, even in the worst cases of juvenile dementia paralytica, are not as severe as those observed in the adult form of the disease. Watson described two degrees of change occurring in the walls of the blood vessels: a chronic reparative process shown chiefly by the hyaline change, and the

other, an active proliferation of much more recent origin. The long strands of the glia cells leading to a capillary to form the sheath of the hemic-encephalic barrier are often seen much more clearly than in preparations from normal brains. Mott [6] especially mentioned the cellular proliferation around the vessels, both in the glia and in the various cellular elements of the vessel. There is an actual increase in the number of blood vessels.

Degenerative Changes.—The most conspicuous degenerative change is the actual reduction in the number of nerve cells in the cortex. Furthermore, these cells are usually disturbed in their position and shape by the great overgrowth of neuroglia tissue. The reduction of cells is most marked in the molecular and granular layers (Stewart [19]) and in general they diminish in number inwardly from the cortex. Alzheimer [4] found a disturbance in the third and fourth layers in one of his three patients, but this was not noted by Mott,[6] Watson [7] or Ricksher [16] or by any other observer. Watson [7] noted a chronic type of degenerative change in the nerve cells in all the cases and an acute type of degeneration of the cells in cases in which the congestion of the vessels was most marked. The relative order of involvement of the different cortical areas was as follows: (1) the prefrontal lobe, (2) the superior and inferior parietal lobules, (3) the Broca convolutions, (4) the central convolutions and (5) the occipital lobe. Watson [7] regarded the slow atrophic degeneration of the nerve cells as the primary and essential change characteristic of juvenile dementia paralytica.

The ganglion cells show fatty changes and various alterations of the chromatin particles and nuclei (Ricksher [16]). Watson [7] especially emphasized the striking absence of pigment in the degenerated cortical nerve cells, which, according to Ricksher,[16] is a change due entirely to age.

The nerve fibers also show changes, although there is some difference of opinion regarding those changes. Watson [7] found a recent degeneration of the radial fibers in practically all the cortical areas; this degeneration was most pronounced in the central and occipital regions, and the afferent tracts were invariably less affected. Mingazzini found that the atrophy of the radial fibers was far more pronounced in juvenile than in adult dementia paralytica and regarded this difference as partly due to the long duration of the disease. Ricksher [16] noted a complete absence of the tangential fibers and, less frequently, a destruction of the radial fibers.

Juba [18] recently described the occurrence of Steiner's myelopholids and colloidal degeneration in two cases of juvenile dementia paralytica, although he stated they are not a part of the characteristic picture. Steiner's myelopholids are spherical bodies which stain intensely with

hematoxylin and are often arranged in rosets. Steiner regarded these bodies as a result of focal destruction of the medullated sheaths by spirochetes, but Juba [18] found them in places in which there was no destruction of the sheaths.

The basal ganglions frequently show severe changes, often more marked than the changes observed in the adult form of dementia paralytica. The degenerative changes of the nerve cells, congestion of the vessels and gliosis were especially mentioned by Watson [7] and Freeman.[22]

In the following case an extreme gliosis of the thalamus was observed.

CASE 42.—The patient was a colored girl, aged 13. The father was unknown; the mother had dementia paralytica, and three other siblings, one of whom was known to be syphilitic, were in a custodial home. The patient had lived in this

Fig. 3.—Photographs of the patient in case 42. At autopsy marked gliosis of the thalamus was noted.

home, but she was referred to the juvenile court for having had sexual relations with a white man. She was sent to the hospital by the court. Physically she was well nourished and showed no gross abnormalities. Her breasts were well developed. The neurologic examination showed Argyll Robertson pupils, tremor of the eyelids, tongue and fingers, a deviation of the tongue to the left, exaggerated reflexes and a stiff ataxic gait. The stigmas present included infantile hands and feet, a hypopituitary habitus and facial asymmetry. Mentally, she was amiable, contented and obedient, but she could not answer even simple questions relevantly or correctly. She probably did not even comprehend questions, generally answering either "yes" or "no." She was restless and often resistive, but she tended to her simple wants and could feed herself.

She was given a course of malaria therapy but continued to deteriorate. A second course was attempted but did not produce any results. She was given several courses of treatment with bismuth and sulpharsphenamine, but without effect. She died at the age of 18 years, after five years in the hospital.

At autopsy the brain was seen to be hypoplastic; on section it showed a deficiency in the subcortical white matter. The basal ganglions were of normal

size. There was only a slight dilatation of the ventricles, but a marked granular ependymitis was observed. The cerebellum appeared normal but was unusually firm. The meninges, particularly the pia, were thickened, and presented a strikingly milky appearance. As observed microscopically the cortex showed a marked reduction in the number of nerve cells and a marked increase in vascularity, with hyperplasia of the endothelium of the vessel walls. There was a marked gliosis throughout, but this was especially marked in the hippocampal area. The thalamus was much more severely affected than in the average case of dementia paralytica; it showed considerable infiltration and thickening of the blood vessels and extreme gliosis. Also in the hypothalamus there were marked infiltration and sclerosis. The aqueduct was broken into a number of different channels by the excessive overgrowth of small glia cells. The ependyma in the lateral ventricles, aqueduct and fourth ventricle was nearly obliterated by the overgrowth of subependymal glia.

Developmental Disturbances.—Many of the changes, both inflammatory and degenerative, have been regarded as developmental disturbances. Ricksher [16] referred to the atrophy and areas of softening in the basal ganglions as suggestive of developmental defects. Juba [18] stated that anomalies of the cerebellum are frequent in this disease; this was also Sträussler's [9] opinion. The latter expressed the belief that juvenile dementia paralytica may occur after 30 years of age, and Lafora [12] maintained that the characteristic developmental (histologic) defects of juvenile dementia paralytica are often seen in adults from 30 to 35 years of age. Furthermore, he stated that in cases in which unquestioned developmental disturbance is evident one is justified in making the diagnosis of late hereditary dementia paralytica.

Changes in the Cerebellum.—The most characteristic structures observed microscopically in the cerebellum in cases of juvenile dementia paralytica are the binucleated and trinucleated Purkinje cells first described by Sträussler [9] (fig. 4). Stewart [19] summarized the present opinion with regard to these cells: Their occurrence, though not confined to patients with juvenile dementia paralytica, must be regarded as highly characteristic of that condition. Their significance is not clearly understood, but it appears probable that binucleated nerve cells are an evidence of some developmental disturbance, and in this connection one may recall the fact that the cyto-architectonics of the cerebellum are still in progress at birth, the final disposition not being completed until the fifteenth month of postnatal life.

The Purkinje cells have been the source of much argument, particularly as to their significance, and yet it is these cells which are chiefly referred to by various writers as evidence of developmental disturbance (Lafora,[12] Ricksher,[16] Bielschowsky,[15] Springlová,[17] Scharnke [14] and Trapet [10]).

Other characteristic changes in the cerebellum include the marked atrophy of the folia, the extensive overgrowth of the microglia, the reduction in the granule layer and the reduction in the white matter (Freeman [22]). Watson [7] mentioned the occurrence of a decided thickening and congestion of the membranes and vessels generally.

Lissauer's Paralysis.—Clinically many cases of juvenile dementia paralytica resemble the picture described by Lissauer, namely, a slowly progressive form of the disease with focal signs such as aphasia or hemiplegia or with repeated epileptiform or apoplectiform attacks. Stewart [19] pointed out that the occasional presence of unilateral atrophy of certain convolutions, together with the microscopic picture of status

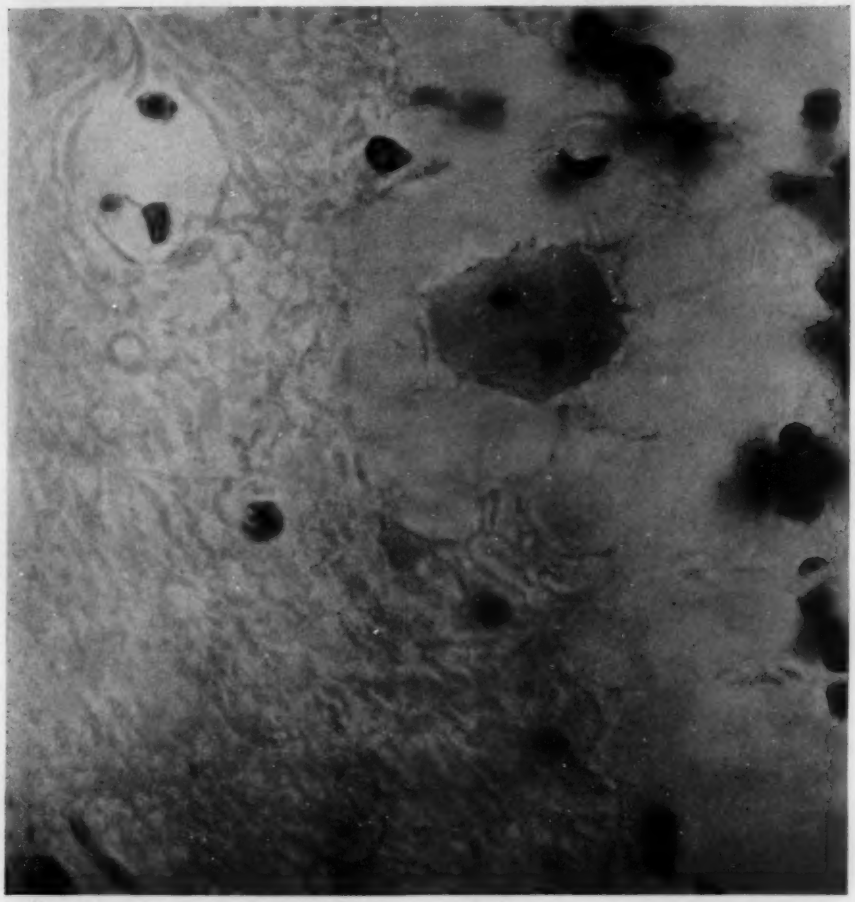

Fig. 4.—Photomicrograph showing binucleate Purkinje cells in the cerebellum in juvenile dementia paralytica.

spongiosus, indicates that there is no sharp dividing line between the pathologic changes of the Lissauer type of dementia paralytica and those which are typical of the juvenile form of the disease.

CHANGES IN THE SPINAL CORD

While juvenile dementia paralytica is to be regarded primarily as an encephalitis, it was pointed out in a previous study that in 62 of

653 cases summarized, the knee jerks were absent. Thus 11.6 per cent of the cases reported as instances of juvenile dementia paralytica are, at least clinically, cases of the tabetic form of the disease. Scharnke [14] is the only writer I have found who mentioned pathologic changes in the spinal cord, and he did so only to point out that many of the ganglion cells are placed in the outer half of the gray substance in the cord. This he regarded as a developmental defect.

In the following case there was sclerosis in the posterior columns and in the pyramidal tracts.

CASE 43.—The patient was a boy, aged 12 years. The father was an alcoholic addict and had syphilis; the mother had acquired syphilis five years before the patient's birth; she died of dementia paralytica after his admission to the hospital. When the patient was 3 months of age he had a syphilitic rash and received treatment. As an infant he was placed in an orphan asylum and grew normally until he was 8 years of age, being bright, active, alert and attentive. Then, without an apparent precipitating cause, he began to grow dull and absent-minded, lost interest in play and failed in his work in school. He began to steal and to lie but showed no marked emotional fluctuations or delusions.

Physical examination showed no gross abnormalities and no stigmas. The neurologic findings included muscular weakness and incoordination. The right pupil was sluggish and the left fixed to light. The patient had a marked defect of speech and of writing. There were gross tremors of the tongue and facial muscles and of the fingers when extended. The deep reflexes, including those of the biceps, triceps, quadriceps and gastrocnemius muscles, were exaggerated. He showed a generalized hypalgesia. Mentally, he was oriented but apathetic, and he was dulled emotionally. He was indifferent to his surroundings and answered questions in a confused manner with many mistakes. The laboratory examination showed a positive Wassermann test of the spinal fluid and blood serum; the spinal fluid contained an increased amount of protein and 123 cells per cubic millimeter.

Occasional convulsive seizures developed, and the patient's mental status showed progressive dementia and deteriorated to a vegetative level. There was a marked reduction in the physical activity, and the patient became emaciated. Bronchopneumonia developed, and he died seven months after admission.

At the postmortem examination the meninges showed marked thickening and a milky appearance. There were marked atrophy, possibly partly hypoplasia, of the brain, and an extensive granulation of the ependyma of the ventricles. The ventricles were slightly dilated. The microscopic picture showed a diffuse inflammatory reaction about the blood vessels, with an infiltration of round cells and an overgrowth of glial cells. There was a general reduction of the nerve elements. Many stab cells were apparent. Binucleated Purkinje cells were numerous in the cerebellum, which also showed marked atrophy and gliosis of the folia. The spinal cord showed sclerosis in the dorsal columns and in the pyramidal tracts, with moderate gliosis and perivascular infiltration around the margins.

This case is specially interesting because of the changes in the spinal cord. The knee and ankle jerks were both exaggerated, and while hypalgesia was present, there were no other signs pointing to the involvement of the cord. Freeman [22] stated that the cord is commonly affected in cases of dementia paralytica, as in this case, without exhibiting focal symptoms that would indicate such degeneration.

SPIROCHETES

Freeman [22] stated that spirochetes are found in about one third of the cases of juvenile dementia paralytica (fig. 5). Stewart [19] commented that the organisms cannot be demonstrated with any more certainty than in the adult form of the disease, that they may be demonstrated

Fig. 5.—Photomicrograph showing spirochetes in the cortex in dementia paralytica (Dieterle impregnation; × 850).

in swarms or that a prolonged search may fail to show any. He stated that the chance for their discovery seems to depend less on the clinical duration of the disease than on the presence of seizures during life, particularly in patients who have numerous seizures before death. Bouman and Bok [25] demonstrated spirochetes lying in the neighborhood

25. Bouman, L., and Bok, S. T.: Nederl. tijdschr. v. geneesk. **68**:1611, 1924.

of the Purkinje cells, and the presence of organisms in the spinal fluid in a case of juvenile dementia paralytica was demonstrated by Marinesco and Minea.[26]

Dieterle[27] made an extensive study of the arrangement and occurrence of spirochetes in a case of juvenile dementia paralytica and noted their presence in great swarms in the principal regions of the cortex, in the basal ganglions and in the cerebellum. In his case the distribution of the spirochetes was primarily perivascular. He found none in the white substance of the convolutions, although great masses of them were observed in the middle zone of the cortex and smaller colonies were noted in the subpial regions. These observations suggested that the spirochetes may have spread via the lymph outward to the arachnoid, although their metastasis over a wider sphere is hematogenous.

SUMMARY

Although there are common observations in the juvenile and the adult form of dementia paralytica, certain features are characteristic of the juvenile form of the disease.

The characteristic macroscopic changes in juvenile dementia paralytica include a generalized atrophy, often hypoplasia of the brain, a marked leptomeningitic reaction, the frequent occurrence of hydrocephalus and an extensive ependymitis granulosa. The cerebellum often shows atrophy. In rare cases there is an associated formation of gummas in the brain. Because of proliferation of neuroglia, both the cerebrum and the cerebellum are usually very firm in consistency.

The characteristic microscopic observations include a generalized round cell infiltration, an extensive proliferation of neuroglia and microglia, an increase in the vascularity with proliferative changes of all the elements of the vessel walls, a reduction in the number of nerve cells in the cortex and changes in the nerve fibers. The frequent finding of binucleated Purkinje cells in the cerebellum is characteristic of, though not limited to, this disease.

A case is cited in which sclerosis of the posterior columns and pyramidal tracts in the spinal cord without symptoms indicating these lesions was observed. One must recognize, however, that hyperactive reflexes can occur if sclerosis of the lateral column predominates over sclerosis of the posterior column.

In a small percentage of cases spirochetes can be demonstrated in the brain, the cortex, the basal ganglions and the cerebellum.

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26. Marinesco, G., and Minea, J.: Compt. rend. Acad. d. sc. **159**:287, 1914.

27. Dieterle, R. R.: Am. J. Psychiat. **7**:547, 1928.

INGUINAL LYMPHOGRANULOMA IN ITS RELATION TO STRICTURE OF THE RECTUM

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CHICAGO

The rôle of inguinal lymphogranuloma in the production of inflammatory stricture of the rectum has been definitely established since the original work of Jersild [1] and of Frei and Koppel.[2] In a recent report [3] we described the postmortem observations in a case of the rectal syndrome of inguinal lymphogranuloma; we now present a report of the pathologic changes in two additional cases of the genito-anorectal syndrome of inguinal lymphogranuloma, with a brief résumé of the previous case.

REPORT OF CASES

CASE 1.—*History and Course.*—A Negress, aged 47, was admitted to the hospital on April 25, 1932. In 1924 she had had a swelling of the left buttock near the anus. The swelling ruptured spontaneously, and a persistent discharging lesion remained. During the following six years many similar lesions, which were occasionally painful, appeared on the left buttock. Two months after her admission the right buttock became involved. She had alternating periods of diarrhea and of constipation, with at times incontinence of feces. There was no pain except when the lesions on the buttocks did not drain properly. She had lost 30 pounds (13.6 Kg.) in weight in about two years; she stated that she had night sweats but did not cough. Roentgenograms of the chest showed evidence of a nonactive apical infection.

There were bilateral swellings of the buttocks, with scattered granulomatous excrescences which varied in size from that of a pea to that of a lemon. A foul-smelling, thick brown discharge drained profusely from the lesions. Grapelike excrescences similar to those present on the buttocks involved the anus and extended into the rectum for the distance reached by the finger. A meal of barium sulphate revealed an apparent rectal stricture. There was a bilateral nontender shotty inguinal adenitis. Smears and cultures of the discharge did not show fungi or pathogenic organisms. Inoculation of guinea-pigs with the pus did not produce evidence of tuberculosis. The Mantoux test (1:10,000) was strongly positive. Repeated Frei tests done with Frei antigens [4] were negative.

From the Dermatological and Pathological Services of the Cook County Hospital and the University of Illinois College of Medicine.

1. Jersild, O.: Ann. de dermat. et syph. **1**:577, 1930; Bull. Soc. franç. de dermat. et syph. **38**:537, 1931.

2. Frei, W., and Koppel, A.: Klin. Wchnschr. **49**:2331, 1928.

3. Wien, Max S., and Perlstein, Minnie O.: M. Rec. **139**:288, 1934.

4. The Frei antigens used were from patients under our observation and from patients of Dr. H. N. Cole, Cleveland.

A Frei antigen prepared from the pus taken from a lesion on the buttock, as suggested by Sulzberger and Wise,[5] gave positive reactions when cross-tested on patients known to have inguinal lymphogranuloma. Microscopic examination of a biopsy specimen from one of the nodules on the buttocks revealed a loose vascular granulation tissue in the corium; this tissue consisted of a fine reticulum with the interspaces filled with plasma cells, leukocytes and oxyphilic granulocytes. A diagnosis was made of rectal stricture, ulcerative proctitis and multiple fistulas of the buttocks due to inguinal lymphogranuloma, with anergy. The patient became more and more cachectic and died on March 29, 1933, one year after admission.

Autopsy.—The autopsy revealed a polyp in the body of the uterus, multiple subserous and intramural fibromyomas of the uterus, fibrous adhesions about both tubes and ovaries, brown atrophy and parenchymatous degeneration of the myocardium, confluent bronchopneumonia of the upper and lower lobes of the left lung, anemia and cloudy swelling of the liver and kidneys, chronic infectious swelling of the spleen with fibrosis, anemia and fatty changes of the liver, multiple ulcerative granulomas about the anus and both gluteal regions, indurative periproctitis with a large abscess to the left of the rectum and multiple fistulous tracts to the rectum, gluteal region and peritoneum.

The mucosa of the rectum (fig. 1) was from purple-pink to pink-gray and slightly edematous. There were numerous openings up to 6 by 5 mm. in diameter surrounded by the rectal mucosa. The fistulous tracts into which these openings led were surrounded by a 3 cm. thick layer of firm whitish scar tissue which surrounded small islands of fat tissue. Anteriorly to the lowermost portion of the rectum, to the left of the vagina, there was an irregular cavity 50 by 40 by 25 mm., lined by transparent dirty purple-gray tissue and communicating through several fistulous tracts with the lowermost portion of the rectum. The scarring along the rectum extended upward for about 10 cm.

Case 2.—*History and Course.*—A Negress, aged 33, was admitted to the hospital on July 9, 1932. The essential point in her history was that she had had a profuse whitish leukorrhea for about ten years which had become fecal in odor and color during the two preceding weeks. In 1928 a "cauliflower-like" growth had been removed from one labium majus. Since 1929 the patient had rectal trouble, especially constipation. During the last three weeks she had severe pain in the rectum with chills and fever. The constipation had been less severe during this time and the stools more watery; at times a fecal discharge occurred through the vagina. She had lost a great deal of weight during the past year.

The right labium majus was enlarged, tender and boggy. The posterior wall of the vagina was fixed, indurated and nodular, and near the introitus was an opening into the rectum from which soft fecal material was discharging. The rectum was tender and fixed. A nodular obstructing mass was present about 4 cm. from the anal orifice.

A diagnosis of carcinoma of the rectum or of the cervix was made, but a biopsy specimen from the nodular area showed a chronic nonspecific inflammation with no evidence of malignant degeneration. The diagnosis of inguinal granuloma was made, and the patient received seven injections of antimony and potassium tartrate without improvement. Her general condition was poor, and she complained of a great deal of rectal pain. There were alternating periods of diarrhea and constipation, with at times bloody stools. Repeated examinations of the stools for pathogenic organisms gave negative results. Serologic tests of the blood were

5. Sulzberger, M. B., and Wise, F.: J. A. M. A. **99**:1407, 1932.

negative. Direct smears and cultures of the purulent secretion did not show pathogenic organisms. On Sept. 22, 1932, a Frei test was done, and forty-eight hours later, a painful papule the size of a hazelnut was present at the site. A diagnosis of rectal stricture, ulcerative colitis and rectovaginal fistula due to inguinal lymphogranuloma was made.

The patient became weaker and died on Oct. 9, 1932.

Autopsy (Dr. R. H. Jaffé).—The mucosa of the large intestine was found to be light pink-gray and edematous, especially in the descending and sigmoid colon. The surfaces of the sigmoid colon were cloudy and studded with superficial

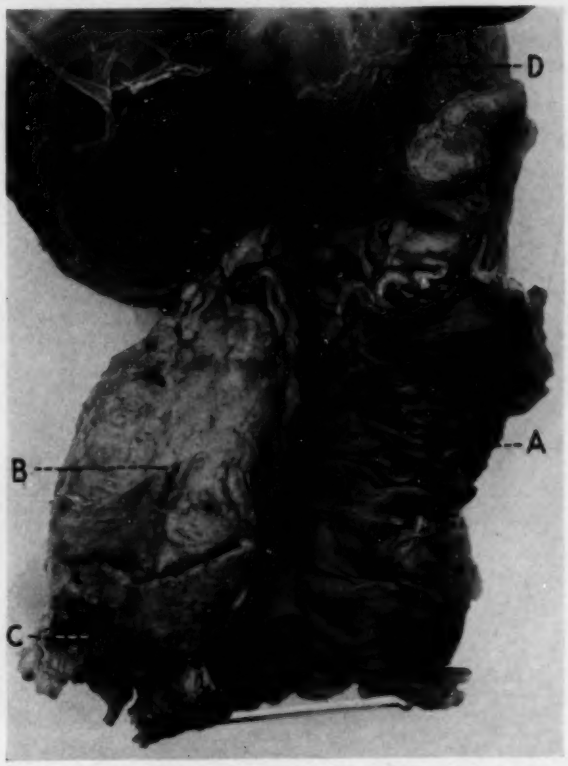

Fig. 1.—Specimen of the rectum and of perirectal tissue showing the absence of rectal stricture. *A* indicates unchanged rectal mucosa; *B*, indurative periproctitis; *C*, an abscess in the perirectal tissue which communicates with the rectum and gluteal region, and *D*, multiple fibroids of the uterus.

depressions up to 15 by 10 mm. in diameter. In the lower half of the rectum were numerous transverse ulcerations separated by swollen and injected mucosa (fig. 2). Some of the ulcers were deep and extended into the perirectal tissue, which was much indurated. To the left of the rectum was a 30 by 60 mm. cavity surrounded by scar tissue and filled with thick dirty yellow-gray pus. The vaginal mucosa was thickened and granular and from purple-gray to purple-red. In the lowermost portion, near the vulva, was an irregular 4 mm. opening which led into

the rectum. About this opening were flat, undermined plaques which ranged in size up to 12 mm. The plaques were covered with the injected mucosa.

The histologic examination of the rectum showed that the floor and walls of the ulcers were lined with cellular and vascular granulation tissue in which small round cells and histiocytes predominated. Plasma cells were scanty. The infiltration extended into the deeper layers of the wall and followed the blood vessels. In the islands between the ulcers the mucosa was thin and cellular. The normal epithelium was replaced by squamous epithelial cells which lined and filled the glands (fig. 3). Many of these epithelial cells were large and clear, and the cell membrane was prominent.

Cultures of material from the ulcers in the intestine showed Bacillus coli and Bacillus pyocyaneus.

The anatomic diagnosis was: ulcerative proctitis and periproctitis with formation of abscesses, rectovaginal fistula, granular colpitis, confluent bronchopneu-

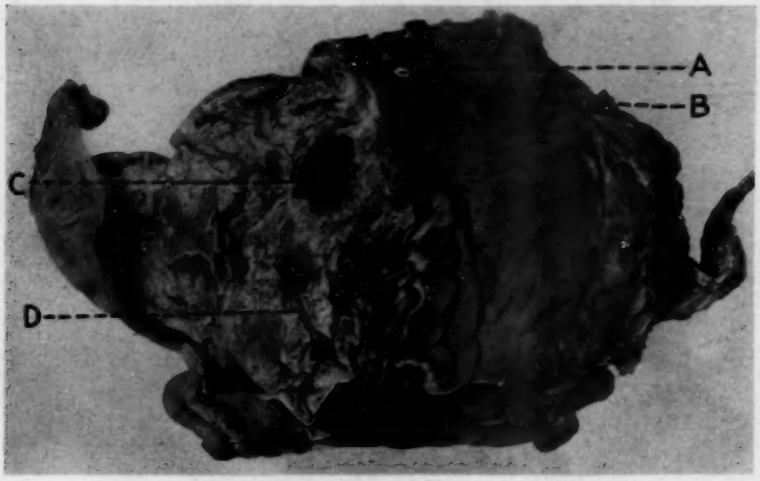

Fig. 2.—Specimen of the rectum and of perirectal tissue showing changes in the distal 10 cm. *A* indicates a rectovaginal fistula; *B*, rectal abscesses; *C*, perirectal ulceration, and *D*, periproctitis.

monia of the lower lobe of the right lung, atrophy and parenchymatous degeneration of the myocardium, anemia and parenchymatous degeneration of the liver and kidneys, chronic infectious swelling of the spleen, fibrous adhesions about the ovaries, tubes, spleen and liver, adenoma of the suprarenal glands and cholelithiasis.

Case 3.—*History and Course.*—A Negress, aged 37, was admitted to the hospital on April 15, 1933, and died on May 5, 1933.

She had been constipated for the past seventeen years, since the birth of her first child. In 1930 she had an "abscess in the rectum;" this ruptured spontaneously and discharged a large amount of pus. This lesion had persisted up to the time of her admission to the hospital. During the previous year she had pain in the rectum and had passed blood and pus in the stool. She had lost about 32 pounds (14.5 Kg.) in weight during the past year.

A diagnosis of venereal stricture was made, and rectal dilations were made at intervals of from one to three weeks. Following the dilations, chills and a rise in temperature up to 102 C. occasionally developed.

In January 1933, a Frei test was positive, and a diagnosis of inguinal lymphogranuloma was made. The dilations were then discontinued for two months. In March 1933, following a dilation, pain developed in the lower part of the abdomen; this pain was aggravated by bowel movements.

There was tenderness over the entire abdomen, especially in the left lower quadrant, where there was a tense, tender mass the size of a hen's egg. There

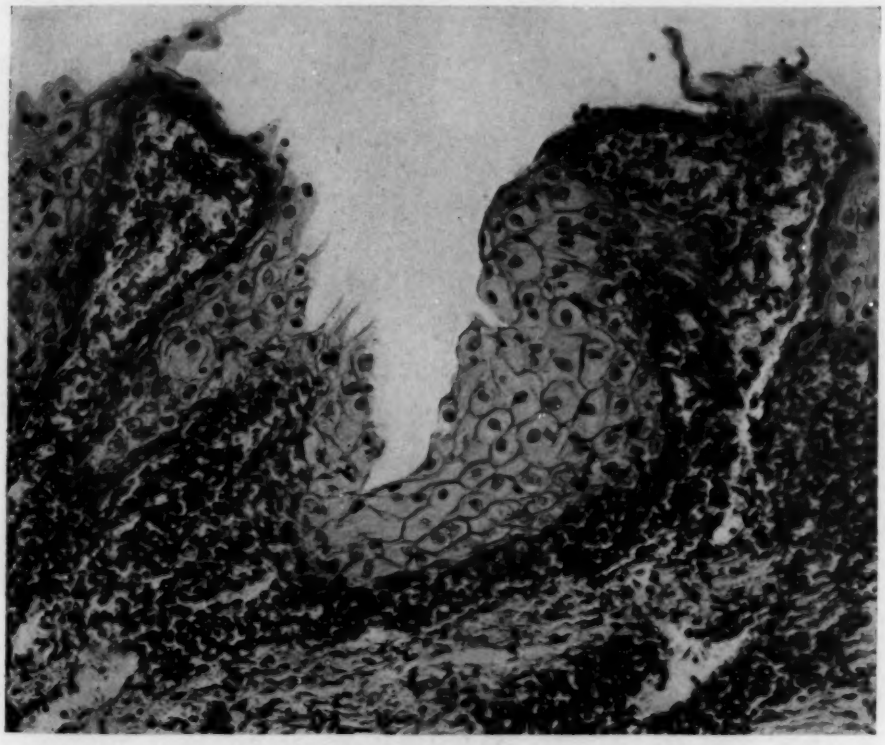

Fig. 3.—Photomicrograph of a section from the mucosa of the rectum showing a metaplasia from columnar to stratified squamous epithelium. (Hematoxylin and eosin; × 220.)

were an infiltration in the right fornix and culdesac and a firm fibrous stricture of the rectum about 2 inches (5.08 cm.) from the anus.

The patient was operated on, and multiple abscesses were found in the pelvic cavity; these communicated with the abscess in the perirectal tissue. Panhysterectomy and salpingectomy were performed. The patient died about two hours after the operation. Frei antigen was prepared from the pus obtained from the abscesses.

Autopsy (Dr. R. H. Jaffé).—The autopsy showed emaciation, passive congestion of the lungs, infectious swelling of the spleen, parenchymatous degeneration of the myocardium, liver and kidneys, evidence of recent surgical removal of the

uterus, fallopian tubes and left ovary and an inguinal lymphogranuloma involving the terminal 9 cm. of the rectum.

The intestinal mucosa was purple-gray, smooth and covered with an increased amount of mucus. Beginning at the mucocutaneous junction of the anus, the rectal mucosa was pink-gray and exhibited folds and bridges. The wall of the rectum in that area was soft. Beginning 3 cm. above the anus the consistency changed

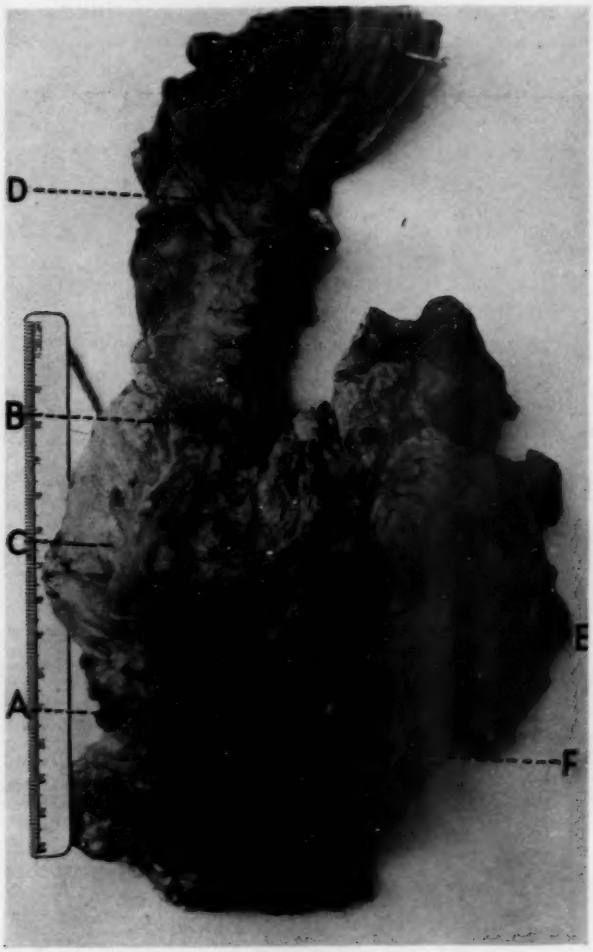

Fig. 4.—Specimen of the rectum and of perirectal tissue showing changes in the distal 10 cm. of the rectum. *A* indicates multiple fistulas in the rectum; *B*, a rectal stricture; *C*, cartilaginous-like changes in the mucosa; *D*, unchanged rectal mucosa; *E*, indurated perirectal tissue, and *F*, an abscess in the perirectal tissue.

and became cartilaginous. The perirectal tissue was indurated and firm, with whitish lines coursing through it. The right ureter was embedded in this perirectal mass but was not obstructed. The mucosa of the rectum at that point and for a distance of 9 cm. was firm, purple-pink and mottled with yellow-green dis-

crete areas, and fistulas led from the perirectal tissue into the lumen of the rectum. The change suddenly ceased at about 13 cm. above the anus, where the mucosa appeared normal (fig. 4).

Histologic examination of the rectum showed that the mucosa was densely infiltrated by plasma cells and lymphocytes between which were found a few large histiocytes and polymorphonuclear leukocytes. The submucosa was much thickened. The arterial walls were thickened, especially the intima, while the small veins were wide and their walls infiltrated by plasma cells and lymphocytes. The muscularis was hypertrophic, and about the large blood vessels there was an increased amount of connective tissue. The capillaries of the muscularis were prominent because of a swelling of their endothelial and adventitial cells. There were a few histiocytes and plasma cells about these capillaries.

RÉSUMÉ OF PATHOLOGIC CHANGES

Gross and microscopic studies of the primary lesion and of the affected lymph nodes in inguinal granuloma have been made by many writers, including Bory,[6] Phylactos,[7] Durand, Nicolas and Favre,[8] Hellerström,[9] Muller and Justi,[10] Hansman [11] and Hoeppli.[12] The lymph nodes were matted together, and small abscesses filled with a yellow-green thick pus were usually seen macroscopically. The nodes showed suppuration and never caseation, thus differentiating themselves from the nodular lesions of tuberculosis. In the main the histologic picture revealed small discrete or ramified abscesses surrounded by zones of epithelioid cells, often in palisade arrangement. In the abscesses there was a granular detritus. The structure of the lymph nodes was almost entirely replaced by a granulation tissue composed of plasma cells, lymphocytes, epithelioid cells and fibroblasts with an occasionally multinucleated giant cell of the Langhans type, usually bordering the epithelioid cells. The cellular infiltration was marked about the lymphatics, and many of the newly formed blood vessels showed some thickening of the walls.

Autopsies on patients with inguinal lymphogranulomas in whose cases antemortem diagnoses have been made are extremely rare. Hillsman, Wilshusen and Zimmerman [13] and Wernich [14] reported an autopsy on a man. In Wernich's case no proctitis was present.

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6. Bory, L.: Bull. Soc. franç. de dermat. et syph. **26**:451, 1931.
7. Phylactos, A.: Lymphogranulomatose des ganglions inguinaux, Thèse de Lyon, 1922; quoted by Hellerström.[9]
8. Durand, M.; Nicolas, J., and Favre, M.: Bull. et mém. Soc. méd. d. hôp. de Paris **35**:274, 1913.
9. Hellerström, S.: Acta dermat.-venereol., supp. 1, 1929, p. 5.
10. Muller, O., and Justi, K.: Arch. f. Schiffs- u. Tropen- Hyg. **18**:857, 1914.
11. Hansman, G.: Surg., Gynec. & Obst. **39**:422, 1924.
12. Hoeppli, R.: Dermat. Wchnschr. **90**:303, 1930.
13. Hillsman, J. A.; Wilshusen, H. F., and Zimmerman, H. M.: Arch. Dermat. & Syph. **18**:383, 1928.
14. Wernich, L.: Zentralbl. f. Haut- u. Geschlechtskr. **24**:137, 1927; Przegl. dermat. **21**:251, 1926.

The gross changes in the rectum usually consist of numerous transverse ulcerations separated by swollen and injected mucosa. Some of the ulcers are deep and extend into the indurated perirectal tissue. Microscopically the mucosa is densely infiltrated by plasma cells and lymphocytes, between which are occasional histiocytes and polymorphonuclear leukocytes. The submucosa is much thickened and fibrosed and contains many perivascular accumulations of plasma cells and lymphocytes. The walls of the smaller arteries, especially of the intima, are thickened, while the small veins are wide and their walls are infiltrated by plasma cells and lymphocytes. The muscularis is usually hypertrophic, and about the larger blood vessels is an increased amount of connective tissue. The capillaries of the muscularis are prominent owing to a swelling of their endothelial and adventitial cells, and about them a few histiocytes and plasma cells are frequently seen. Occasionally, the normal epithelium undergoes a metaplasia to a squamous epithelium, as was present in case 2 (fig. 3). This squamous epithelium lined and filled the glands. Many of the epithelial cells were large and clear, with prominent cell membranes. Plasma cells were scanty. The infiltrations extended into the deeper layers of the wall and followed the blood vessels. In the islands between the ulcers, the mucosa was thin and cellular.

COMMENT

The clinical evidence of the rôle of inguinal lymphogranuloma in inflammatory rectal stricture has been thoroughly reviewed by Stannus [15] and further emphasized in the recent report of Bloom.[16] Some proctologists have reluctantly accepted this conception, as evidenced by the statement of Rankin, Bargen and Buie.[17] Rosser,[18] however, said: "The mass of recent literature . . . makes it impossible to disregard the possibility that some obliterating inflammatory rectal strictures . . . must be attributed to this disease." He concluded with the suggestion, however, that ". . . complete abandonment of all previous theories is premature from the evidence."

On the basis of our observations, which are in accord with the reports in the literature, it may be concluded that the anorectal component is an inevitable sequel in both men and women when the perirectal glands are involved either by direct or by retrograde extension of the infection.

Postmortem examinations in cases in which the diagnosis of inguinal lymphogranuloma was made antemortem have been rare. To our knowl-

15. Stannus, H. S.: A Sixth Venereal Disease, Baltimore, William Wood & Company, 1933.

16. Bloom, D.: Surg., Gynec. & Obst. **58**:827, 1934.

17. Rankin, F. W.; Bargen, A. J., and Buie, L.: The Colon, Rectum and Anus, Philadelphia, W. B. Saunders Company, 1932.

18. Rosser, C.: J. A. M. A. **101**:1043, 1933.

edge, the cases described here are the first instances reported in which the necropsy substantiated the clinical diagnosis of inguinal lymphogranuloma in women.

In case 1 roentgenograms revealed an apparent rectal stricture, but no such stricture was noted at autopsy, the stricture in this instance no doubt being of a spastic variety secondary to the rectal and perirectal inflammation. In addition this patient had a dissecting cellulitis of the buttocks due to an extension of the infection from the glands of Gerota to the involved skin. The Frei test was negative, but a positive reaction was obtained by cross-testing.[3]

In case 2 there were a rectovaginal fistula, ulcerative colitis and a rectal stricture that suggested a malignant involvement of the pelvic organs with metastases. On postmortem examination a large perirectal abscess surrounded by scar tissue and containing thick, dirty, yellow-gray pus was found. The rectum was markedly constricted, and deep ulcerations extended into the perirectal tissue.

In case 3 there was a rectal stricture associated with a periproctitis and adhesions and fibrosis about the fallopian tubes and ovaries that suggested a gonorrheal salpingo-oophoritis. Following rectal dilation for a presumed venereal (gonorrheal?) stricture there developed acute abdominal pain with fever and a mass in the left fornix with associated filtration in the culdesac.

In this case an operation was made to drain the abscess, and the firm adhesions about the wall were separated from the surrounding tissue with difficulty. Frei antigen was prepared from the pus indicating the specificity of the exudate in the perirectal abscess in this patient. The Frei test was positive in cases 2 and 3.

The perirectal tissue in these cases was markedly indurated, indicating a periproctitis of long standing. The perirectal changes appeared to have antedated the changes in the rectum, and this fact indicates that the proctitis was secondary to the periproctitis. Since the inflammatory cells were frequently perivascular, the infection had evidently spread by way of the perirectal lymphatics.

In addition to the usual direct spread, along the course of normal drainage of the lymphatics, as summarized by Bloom,[16] retrograde extensions through the lymphatics may occur. A retrograde extension through the lymphatics involving the lymphatics of the penis has been described by Nicolau and Banciu,[19] by Lohe and Rosenfeld[20] and by Goldstein and Byars.[21]

In case 3 a retrograde extension of the infection to the lymphatic nodes of the pelvis would account for the panmetritis associated with periproctitis.

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19. Nicolau, S., and Banciu, A.: Ann. de dermat. et syph. **3**:332, 1932.
20. Lohe, H., and Rosenfeld, H.: Med. Klin. **28**:1485, 1932.
21. Goldstein, D. W., and Byars, L. T.: J. Arkansas M. Soc. **30**:192, 1934.

We now have under observation a Negress with the genito-anorectal syndrome who shows arrested axillary involvement and postauricular fluctuant adenitis. In a white man with inguinal lymphogranuloma involving the inguinal lymphatics there developed a suppurative cervical adenitis. Frei antigen was prepared from the pus obtained from the suppurating glands from both patients.

These observations tend to indicate that we are dealing with a lymphatic-borne disease which may involve any gland in the lymphatic system either by direct or by retrograde extension.

SUMMARY

The clinical histories, the results of autopsy and the microscopic observations for three Negro women with inguinal lymphogranuloma are presented.

The first patient had a dissecting cellutitis of the buttocks. The autopsy showed a fibrosis and chronic inflammation of the perirectal tissue.

The second patient presented the symptoms of a maligant involvement of the rectum with ulcerative colitis. The autopsy showed a combined rectal and perirectal inflammation with multiple rectal fistulas and a rectal stricture.

The third patient presented an acute salpingo-oophoritis associated with a rectal stricture. At autopsy there was found a rectal stricture with periproctitis and multiple abscesses and fibrosis about the fallopian tubes and the ovaries.

The patients presented a uniform microscopic picture of fibrosis and plasma cellular infiltrations which extended from the perirectal tissue to the mucosa of the rectum. In one of the patients there was a metaplasia of the columnar epithelium of the rectal mucosa to stratified squamous epithelium.

The terminal stage of the genito-anorectal syndrome of inguinal lymphogranuloma, together with its varied associated clinical manifestations, is well illustrated by the reports here presented. The postmortem investigations furnish the anatomic evidence for the pathogenesis of the condition, and they agree with the numerous clinical deductions detailed in the existing literature.

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EXPERIMENTS ON COMPENSATORY RENAL HYPERTROPHY

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The fact that a kidney enlarges after its fellow has been destroyed by disease or removed by operation has been known for about a century. During this period extensive anatomic investigations have clearly shown (1) that the enlargement takes place as a result of growth by hypertrophy, an increase in size, of glandular elements of the kidney, that is, of glomeruli and tubules, and (2) that the formation of new glomerulotubular units, growth by hyperplasia, ceases at or soon after birth. Crucial experiments on very young mammalian animals have verified the fact that the enlargement of a remaining kidney is due solely to hypertrophic changes, no new tubules being formed even at an early postnatal age.

The conception that this process is a compensatory one by which the body attempts to make up for the loss of part of its renal tissue is generally accepted. The phrase "compensatory renal hypertrophy" expresses the idea and is universally used to designate this singular phenomenon.

Most investigations on restorative properties of tissue have been conducted on invertebrate animals. These investigations show that in general among the more primitive animals entire organs as well as a portion of the body may be wholly regenerated after serious injury or complete loss, whereas among higher forms of animals, the vertebrates, replacement occurs, if at all, by growth of surviving fractions of the injured part—not by the formation of a new organ.

Mammals, as invertebrates, are subject to environmental injuries, and their survival is similarly dependent on their capacity for adjustment and repair. Although little is known about the physiology of restorative processes in man, physicians have learned to promote healing by taking advantage of the adjusting and reparative properties of tissue. The knowledge that a subject can survive without one kidney, for example, while commonplace today, was a notable contribution to the medical science of the nineteenth century. From this beginning has grown a large body of effective surgical practice in the treatment of certain

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renal diseases. In great part this practice rests on the capacity of the body to adjust itself to the loss of large fractions of renal tissue.

While the fact that the remaining kidney enlarges after the removal of its mate has been known for a long time and is generally recognized as being of considerable clinical importance, the cause or causes for this hypertrophy and the mechanism producing it are not fully understood. The most satisfactory working hypothesis as to the cause of the phenomenon of compensatory renal hypertrophy is that it constitutes a physiologic or work hypertrophy. When one kidney becomes functionless, from whatever cause, its fellow must do more work in excreting from the blood all instead of half of the constituents of the urine. The solitary kidney responds by a gradual enlargement of its glandular elements, the glomeruli and tubules, this enlargement resulting finally in an increase in mass, or compensatory hypertrophy, of the kidney.

We had three objectives in making this series of investigations: (1) to test further the hypothesis that compensatory hypertrophy of the

TABLE 1.—*Increase in Weight of Remaining Kidney (Morel and Verliac)*

Phase	Time Interval, Days	Increase in Weight, Percentage
1	0 to 10	32 to 37
2	10 to 20	9 to 16
3	20 to 50	11 to 16

kidney is due to increased work; (2) to determine whether some of the constituents of the urine were more effective than others in causing the hypertrophy, and (3) to attempt to find substances and procedures which would hasten the process of hypertrophy.

GENERAL METHODS

In general the plan of our research consisted in excising the right kidney of all the animals of a given litter, with the exception of certain control animals, and then dividing the litter into two fairly equal groups, one group to be observed as a control and the other to be used for experimental purposes. Animals in the experimental group were then subjected to various procedures. After an appropriate interval the entire litter was killed; quantitative observations were made on the remaining kidney, and the observations on the experimental group were compared with those on the control group. Certain of the experiments were prolonged for some months, and animals were killed at intervals in order that a curve showing the rapidity and limits of hypertrophic growth might be obtained. All the investigations reported in this paper were carried out on rats and rabbits.

Albino rats proved admirably adapted to the purpose of the experiments because: (1) they could be had in larger numbers and were well acclimated to laboratory conditions, (2) large litters provided opportunity for control observations, (3) compensatory renal hypertrophy in this species is complete in about six weeks, and its normal development has been thoroughly studied, (4) albino rats withstand operation well and (5) being omnivorous they readily adjust themselves to marked alterations in diet, an important attribute for some of the experiments.

The colony of albino rats from which the rats for these experiments were taken has been maintained for about ten years. The parent rats were obtained from the Wistar Institute of Philadelphia, and there have been occasional additions of new stock during this period. A well balanced commercial ration of ground food or biscuit, which is designated as the standard ration, has been used at the Institute as a standard diet for rats for several years. On this diet the animals grow normally, and deficiency diseases do not develop.

Animals in the adolescent period of growth were used. Most experiments were begun, with nephrectomy, when the litter had reached from 8 to 10 weeks of age and terminated eight weeks later. Certain experiments were prolonged for from four to six months. Since in rats, as in most mammals, the right kidney is slightly larger than the left, the former was selected for nephrectomy in all the experiments. Thus the left, or remaining, kidney, being smaller, had to overcome this difference before a gain in mass over its fellow, in the form of hypertrophy, was evident, whereas had the left kidney been removed, the right, being larger, would have been credited with an increase in mass over its fellow when hypertrophy had not even begun. The absolute and relative increase in the mass of the hypertrophied left kidney over that of the excised right kidney is, therefore, actually greater than the results indicate.

All the operative procedures were carried out with the animal under ether anesthesia, and the usual surgical technic was employed. All the animals used in one series of experiments were operated on at the same period. Immediately after removal each kidney was placed in an individual bottle containing a dilute solution of formaldehyde, U. S. P. (1:10). It is well known that a delay of even a few minutes in the fixation of glandular tissue such as that of the kidney permits cytologic changes to occur that make later microscopic observations unreliable. When a large number of operations had to be performed the danger of delay was too great to permit measurement of the weight of the fresh kidney. However, since all the kidneys, normal and hypertrophied, were treated similarly, the slight change in weight due to the solution of formaldehyde should be relatively the same and consequently immaterial to the results because, as will appear later, all the conclusions are based on a comparison of differences between the relative hypertrophy in control and that in experimental animals.

At the conclusion of the experiment, that is, after eight weeks or longer, the hypertrophied left kidney was removed with the animal under light ether anesthesia and placed in a diluted solution of formaldehyde U. S. P. (1:10), after which the animal was killed and a general inspection of the body was made for evidence of disease. The weight of the body and pertinent observations were recorded.

After being fixed in solution of formaldehyde for from two to three days, each kidney was weighed on a balance which measured accurately to 1 mg. The volume

was measured by noting the amount of water which the kidney displaced when it was introduced into a chemical graduate of appropriate size (10, 20 or 30 cc. capacity), a measurement which was accurate to 0.1 cc. The length, width and thickness of the kidney were measured with a pair of mechanical dividers. The distance between the two points of the dividers representing the maximal length, width and thickness was measured in centimeters. The thickness of the cortex and of the medulla was also measured on the surface of a cross-section of the kidney made at a point midway between the poles.

In certain experiments the diameters of ten renal corpuscles and of ten convoluted tubules were measured with a filar screw micrometer, an instrument which measured accurately to 1 micron. These measurements were made from thin (from 7 to 8 microns) mounted sections stained with hematoxylin and eosin which were cut from the middle portion of each kidney. The structures for measurement were taken at random, except that only the corpuscles which happened to be sectioned through the pedicle in such a fashion as to bisect the glomerulus and only the convoluted tubules which were cut squarely across were measured. The diameter of the corpuscle represents the greatest distance between opposite sides of the outer layer of Bowman's capsule. Observations on pathologic changes were also recorded.

In certain experiments it was desired to have a larger animal than the rat, and in these experiments rabbits were used. On the whole, the rabbits were found to be inferior to the rats for most purposes of this investigation. The general procedure was carried out on the rabbits in the manner described for the rats.

In some of the experiments it was essential to make analyses of the food and urine. These analyses included determinations of the intake of total nitrogen and water and of the total urinary volume and output of nitrogen and general qualitative urinalysis.

A special cage was built for the purpose of measuring the intake of food and water and of collecting urine over a period of twenty-four hours. The urine was collected under oil to prevent evaporation. A small loss of urine, resulting from adhesion to the collecting funnel, was unavoidable. This error is self-correcting, since it was a constant factor in all the experiments. The conclusions are drawn from comparisons of the total urinary output under different experimental conditions and not directly from the absolute values which are involved.

All the determinations of total nitrogen were made by the standard Kjeldahl method.

The general urinalysis included observations of the total quantity, specific gravity (urinometer method) and reaction (acid or alkaline), Benedict's qualitative test for sugar, a qualitative test for albumin with a rough quantitative estimate (grades 1 to 4) and microscopic examination of the sediment for casts, erythrocytes, leukocytes and crystals.

All the quantitative observations, namely, those on weight, volume and linear measurements, were recorded in tabular form, classified according to experiment and subdivided into control and experimental groups appropriate for comparison. The increase in body weight and the increase in the weight of the hypertrophied left kidney during the period of the experiment over the weight of the excised right kidney was computed in grams and in percentage and tabulated. The relation of the weight of the hypertrophied kidney to the body weight was determined by dividing the weight of the kidney in grams by the weight of the body in kilograms. The index number thus obtained represents the number of grams of renal tissue for each hypothetic kilogram of body weight.

Biologic data generally are subject to variations which are uncontrollable. This factor of variability can be obviated somewhat by making a large series of observations from which a normal frequency distribution curve can be made, but it was impossible to compute such a curve in our experiments because large numbers (hundreds) of animals were not available. However, by using litter mates for each experiment, the age factor as a variable was eliminated. Furthermore, it is fair to believe that there is less likelihood of wide variation between litter mates than between animals of the same age but of different litters, although it is no doubt true that, in general, a given litter may be subject to as great variations as the stock from which it is derived. Fortunately, the range of variability of values for the weights of hypertrophied kidneys in the experimental animals, when at all significant, did not fall within the range of variability for values of the litter mate controls. In some experiments the ranges of variability of the experimental and control groups overlapped, in which event the results were considered inconclusive and were so designated.

SERIES 1. EFFECT OF A DIET RICH IN PROTEIN AND OF A DIET CONTAINING UREA ON COMPENSATORY HYPERTROPHY OF THE KIDNEYS OF RATS

The first series of experiments dealt with the question of whether or not the excretion of an increased amount of nitrogenous constituents in the urine would increase the rate and maximal development of the hypertrophy of the remaining kidney.

Experiment 1A. Effect of a Diet Rich in Protein Fed for Eight Weeks.—Since casein is used widely as a convenient protein for experimental dietary mixtures and since Newburgh and Johnston[1] have shown that this substance, unlike foods containing nucleoproteins, does not injure the kidneys, a fact which is corroborated in the present experiments, we employed this substance to furnish the increase in protein of the diet desired in these experiments.

A mixture of equal parts by weight of powdered casein and the standard ration was used. Thus, since the standard ration contained not less than 20 per cent of protein (table 2), the total protein content of the diet was not less than 60 per cent. A quantitative analysis showed that 1 Gm. of this mixture contained, on the average, 82.57 mg. of nitrogen, while 1 Gm. of the standard ration contained 24.35 mg. (table 3).

The experimental animals of the litter of rats, including one animal which was not operated on, received the high protein diet on the day following the unilateral nephrectomy and subsisted on it alone for the

1. Newburgh, L. H., and Johnston, M. W.: J. Clin. Investigation **10**:153, 1931.

duration of the experiment (eight weeks). The control animals, including the one animal which was not operated on, were fed the standard ration for the duration of the experiment.

TABLE 2.—*Food Elements and Ingredients of Standard Ration* *

Food Elements	Percentage, Not Less Than
Crude protein	20
Crude fat	3
Carbohydrates	54
Crude fiber	4
Nitrogen-free extract	50

* These food elements are derived from dried meat, corn grits, ground oat groats, germ of wheat kernel, barley malt, molasses, dried buttermilk, and cod liver oil. Iodized salt, 1 per cent by weight, is added.

TABLE 3.—*Total Nitrogen Content of Various Food Mixtures*

Analysis	Total Nitrogen in 1 Gm. of Various Food Mixtures		
	Standard Ration, Mg.	Standard Ration and Casein (50 per Cent), Mg.	Standard Ration and Urea (20 per Cent), Mg.
1	24.00	83.71	110.83
2	25.96	82.47	114.80
3	23.08	81.53	108.07
Mean values	24.35	82.57	111.23

TABLE 4.—*Comparison of Renal Weight and Body Weight of Animals in Experiment 1A, Series 1*

	Rat Number	Rat Number in Series*	Sex	Increase in Body Weight		Increase in Renal Weight		Relation of Renal Weight to Body Weight (Index Number)
				Grams	Percentage	Grams	Percentage	
Control portion of litter, on balanced diet	1	2	F	59.2	97.3	0.55	137.5	7.92
	2	2	M	81.0	142.1	0.44	114.4	5.91
	3	7	F	69.0	87.3	0.69	133.9	8.28
	4	8	M	76.4	90.3	0.70	126.3	7.29
	Means			71.4	104.2	0.59	128.0	7.35
	Ranges			59.2 to 81.0	87.3 to 142.1	0.44 to 0.70	114.4 to 137.5	5.91 to 8.28
Experimental portion of litter, on high protein diet	5	10	M	110.0	94.1	(Control, no operation)		8.77
	6	11	F	54.0	71.1	(Control, no operation)		11.38
	7	1	F	50.0	71.4	0.80	212.0	9.75
	8	4	F	40.0	47.0	0.78	164.0	10.00
	9	5	M	79.5	127.2	1.00	245.3	9.98
	10	6	M	60.0	91.8	1.16	216.8	9.02
	11	9	F	39.2	51.7	0.74	159.8	10.39
	Means			59.8	75.8	0.90	199.6	9.83
	Ranges			39.2 to 90.0	47.0 to 127.2	0.74 to 1.16	159.8 to 245.3	9.02 to 10.39

* These numbers appear in figure 1.

Results: The result of this experiment (fig. 1; table 4) showed that the hypertrophied kidneys of the animals on the high protein diet were larger than the hypertrophied kidneys of the animals on a standard diet. The magnitude of the difference is indicated by comparing the increase in weight of the left kidney with that of the excised right

kidney; the difference is, on the average, 199.6 per cent for the group which received food with a high protein content and 128 per cent for the control group, and the largest kidney observed in the control animals was smaller than the smallest hypertrophied kidney in the experimental animals.

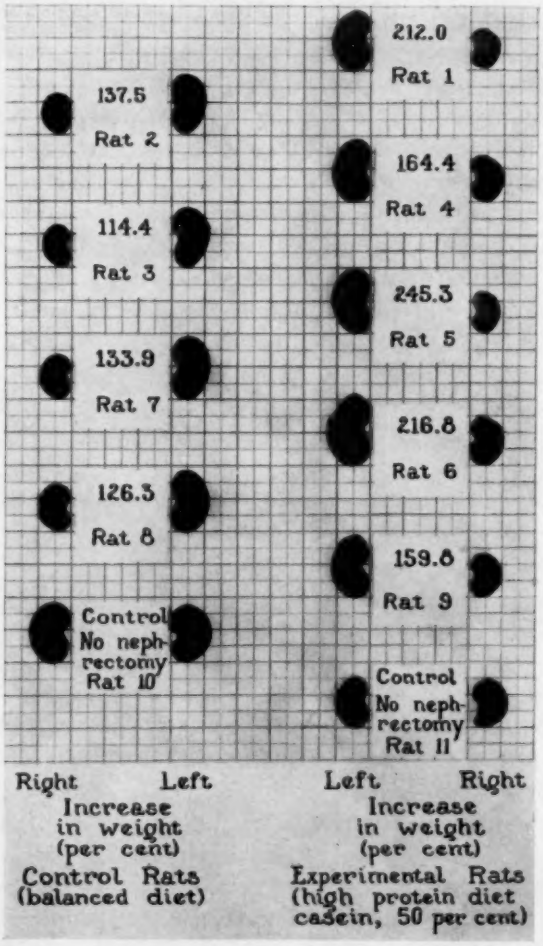

Fig. 1 (experiment 1A, series 1).—Kidneys from albino rats showing the effect of a diet rich in protein containing 50 per cent of casein fed for eight weeks after excision of the right kidney.

That this observation is not due to differences in growth of the rats is evident from a comparison of the index numbers which represent the amount of renal tissue per unit of body weight: 7.35 for the control animals and 9.83 for the experimental animals. In the case

of the animals which were not nephrectomized, the index numbers are 8.77 for the control group and 11.38 for the experimental group, which suggests that the high protein diet also stimulated bilateral hypertrophy. The fact that the percentage of increase in body weight for the experimental group is 75.8 as compared with 104.2 for the control group also indicates that the influence of growth on the body weight was insignificant.

The external dimensions, the length, width and thickness, of the hypertrophied kidneys increased in about the same ratio; hence it is evident that the contour of the kidney remained roughly the same. The cortex and medulla increased in thickness; the increase was more marked in the experimental animals, and in these animals the medulla apparently underwent the greatest change. Since, however, renal growth takes place in three dimensions and is not a purely linear function, this observation does not mean that the greatest amount of growth actually occurs in the medulla. It does suggest, nevertheless, that a considerable increase in the length of collecting tubules (medullary structures) occurs.

Experiment 1B. Effect of a Diet Rich in Protein Fed for Eight Months.—The same diet rich in protein was used as in the previous experiment. This experiment differed from the previous one in several respects: (1) It was prolonged to eight months; (2) control observations were made on animals of the same age but of different litters, some of which were not subjected to unilateral nephrectomy; (3) animals were killed at intervals; (4) estimate of the total intake of nitrogen and of the urinary output of nitrogen were made, and (5) microscopic studies were made, which included measurements of convoluted tubules and renal corpuscles.

Results (figs. 2, 3, 4 and 5; tables 5 to 9): Data on the relation between the total weight of the kidney and the weight of the body during the period of growth of albino rats were not available. Since this information was necessary to a proper interpretation of the present experiment, in the first control group litters of rats of the same age were killed at intervals, and the necessary observations were made. These observations indicate (fig. 2; table 5) that the index number representing the amount of renal tissue per unit of body weight decreases gradually from about 10 at sixty days to about 6 at one hundred and twenty-five days and then increases to 10 at two hundred and twenty-five days. In other words, during this period, which was roughly the period of the experiment, the kidneys do not grow at the same rate as the body; at first the body grows faster, and later the kidneys grow faster. This fact can be shown graphically.

The second control group consisted of two litters of rats which underwent excision of the right kidney. The index numbers were small

at first, but as compensatory renal hypertrophy became more marked the numbers became larger, and therefore the curve (fig. 2, table 6) describes an ascending course. It should be noted, however, that in the animals of this group complete compensation for the loss of one kidney never occurred during the time of observation, as the curves do not meet but toward the end parallel each other.

The curve described by the index numbers (table 7) of the unilaterally nephrectomized animals on a high protein diet takes a course quite different from that of the curves for the two control groups. It rises sharply at first, indicating that the kidney is growing much faster than the body, and early in the course it crosses the curve for the

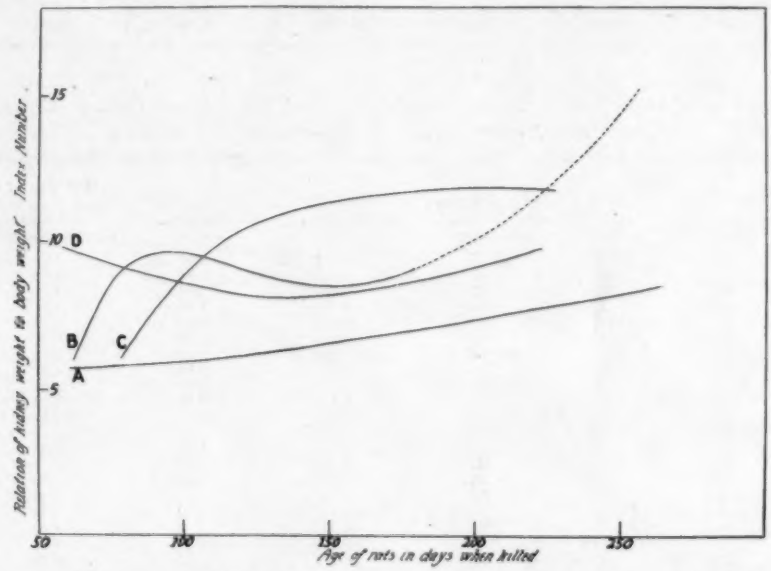

Fig. 2 (experiments 1B and 2B, series 1).—Comparison of smoothed curves, showing the relation of renal weight to body weight of albino rats under control and experimental conditions at various ages. *A* is the curve for the weights of the hypertrophied kidneys of unilaterally nephrectomized rats; *B*, for the weights of the kidneys of rats receiving a high protein diet (experiment 1B); *C*, for the weights of the kidneys of rats receiving a mixture of the standard ration and urea (experiment 2B), and *D*, for the combined weights of the kidneys of normal rats.

control groups, but it soon levels off (at one hundred days), to parallel the latter curve and finally ascends again (a huge kidney [index number 14.36] developed in one animal after one hundred and eighty-eight days on the diet). It is quite evident that the effect of the high protein diet has been to increase the relative amount of kidney tissue far above that which would normally have developed. Also it appears that the limit of capacity for hypertrophy is very great indeed.

TABLE 5.—*Comparison of Renal Weight and Body Weight of Control Animals in Experiment 1B, Series 1*

Rat Number	Sex	Age When Killed, Days	Body Weight, Kg.	Combined Weights of Kidneys, Gm.	Relation of Renal Weight to Body Weight (Index Number)
12	M	61	0.1480	1.48	9.97
13	M	61	0.1500	1.28	8.43
14	F	61	0.1080	1.03	9.54
15	F	68	0.1120	1.22	10.92
16	F	75	0.1420	1.24	8.77
17	M	82	0.1840	1.70	9.21
18	M	82	0.1980	1.84	9.29
19	F	82	0.1200	1.17	9.73
20	F	89	0.1530	1.30	8.46
21	M	93	0.2210	1.88	8.99
22	F	96	0.1480	1.37	9.26
23	F	103	0.1550	1.10	7.06
24	F	110	0.1560	1.13	7.24
25	M	114	0.2325	2.40	10.30
26	F	117	0.1620	1.10	6.79
27	F	124	0.1500	1.38	9.20
28	F	131	0.1540	1.42	9.19
29	M	135	0.2610	1.90	7.41
30	F	217	0.1490	1.48	9.93

TABLE 6.—*Comparison of Renal Weight and Body Weight of Control Animals (Unilateral Nephrectomy) of Experiment 1B, Series 1*

Rat Number	Sex	Age When Killed, Days	Body Weight, Kg.	Renal Weight, Gm.	Relation of Renal Weight to Body Weight (Index Number)
31	M	70	0.172	0.90	5.20
32	M	77	0.128	0.84	6.52
33	F	77	0.145	1.02	7.00
34	M	84	0.186	0.92	4.97
35	F	91	0.144	0.96	6.63
36	F	91	0.133	0.96	7.22
37	M	98	0.228	1.46	6.38
38	M	105	0.190	0.94	4.97
39	F	105	0.135	0.86	6.33
40	F	119	0.144	0.86	6.14
41	F	119	0.147	0.72	4.93
42	M	133	0.243	1.58	6.50
43	M	133	0.141	1.02	7.27
44	F	147	0.174	1.17	6.72
45	M	147	0.138	0.92	5.79
46	M	175	0.206	1.33	6.46
47	M	175	0.148	1.22	8.24
48	F	215	0.192	1.28	6.69
49	M	255	0.198	1.36	6.89
50	F	255	0.164	1.69	10.30

TABLE 7.—*Comparison of Renal Weight and Body Weight of Experimental Animals (on High Protein Diet) of Experiment 1B, Series 1*

Rat Number	Sex	Age When Killed, Days	Body Weight, Kg.	Renal Weight, Gm.	Relation of Renal Weight to Body Weight (Index Number)
51	F	68	0.120	0.83	6.92
52	M	75	0.143	1.29	8.36
53	F	82	0.116	1.11	9.57
54	F	89	0.100	1.08	10.75
55	M	96	0.145	1.15	7.93
56	F	103	0.108	0.94	8.70
57	M	110	0.179	1.55	8.66
58	F	117	0.140	1.31	9.36
59	M	124	0.185	2.20	11.87
60	F	131	0.156	1.12	7.12
61	M	138	0.260	1.70	6.52
62	F	145	0.146	1.30	8.87
63	M	173	0.192	1.58	8.26
64	M	250	0.189	2.72	14.36

Quantitative chemical studies (table 8) showed that the average twenty-four hour intake of total nitrogen of ten rats (five normal and five unilaterally nephrectomized animals) on the standard diet was 263

TABLE 8.—*General Data on Intake of Food and Water and Output of Urine in Control, Unilaterally Nephrectomized and Normal Animals on a Standard Diet and in Experimental Animals on a High Protein Diet and on a Diet Containing Urea (Experiments 1B and 2B, Series 1)*

Rat Number	Experiment	Sex	Body Weight, Gm.	Twenty-Four Hour Intake of: Food, Gm.	Total Nitrogen Mg.	Water, Cc.	Twenty-Four Hour Output of: Urine, Cc.	Total Nitrogen, Mg.	Albumin Content of Urine, Grade	Results of Microscopic Examination of Urinary Sediment
Unilaterally Nephrectomized Animals on Standard Diet										
34	1-B 2-B	M	152.0	7.5	182.6	10.0	4.0	136.4	0	Negative
46	1-B 2-B	M	168.0	10.0	243.5	17.5	5.0	140.5	1	Negative
50	1-B 2-B	F	149.0	15.0	365.2	23.0	6.0	140.3	—1	Negative
41	1-B 2-B	F	141.0	9.5	231.3	16.0	5.0	155.6	1	Negative
45	1-B 2-B	M	151.0	12.0	292.2	21.0	6.0	148.8	1	Negative
Means.....				10.8	263.0	17.5	5.2	144.3		
Normal Animals, No Nephrectomy, on Standard Diet										
13	1-B 2-B	M	140.0	10.0	243.5	15.0	6.0	145.8	—1	Negative
65	1-B 2-B	M	158.0	9.0	219.2	17.0	6.0	133.4	1	Negative
27	1-B 2-B	F	145.0	12.5	304.4	11.0	4.0	140.8	1+	Negative
20	1-B 2-B	F	142.0	9.5	231.3	20.5	6.0	138.2	0	Negative
25	1-B 2-B	M	195.0	14.0	340.9	24.5	7.0	160.6	—1	Negative
Means.....				11.0	267.8	17.6	5.8	143.8		
Animals on a High Protein Diet										
60	1-B	F	143.0	20.0	1,651.4	47.2	17.5	613.20	2	Moderate number of hyaline casts and red blood cells; no white blood cells
61	1-B	M	205.0	21.0	1,734.0	53.0	18.0	749.34	2	
63	1-B	M	175.0	18.0	1,486.3	45.0	16.0	614.40	1	
62	1-B	F	138.0	23.0	1,899.1	42.0	15.0	648.20	2	
Means.....			165.2	20.5	1,692.7	46.5	16.6	656.28		
Animals on a Diet Containing Urea (20 per Cent)										
66	2-B	M	141.0	12.0	1,334.8	35.0	18.0	496.80	2	Few hyaline casts and very few red blood cells; no white blood cells
67	2-B	F	144.0	13.0	1,445.1	85.0	50.0	1,244.90	1	
68	2-B	F	152.0	20.0	2,224.6	57.0	52.0	1,078.48	1+	
69	2-B	M	136.0	15.0	1,668.0	48.0	43.0	1,075.00	1	
Means.....			143.2	15.0	1,668.4	56.2	43.0	973.80		

and 267.8 mg., respectively. The intake of water during the same period was 17.5 cc. The output of urine amounted to 5.2 cc. and 5.8 cc., which contained 144.3 and 143.8 mg., respectively, of nitrogen. The figures for the intake and output for the two control groups

(normal and nephrectomized animals) were substantially the same. Four of the animals on the high protein diet, however, ate, on the average, 20.5 Gm. of food containing 1,692.7 mg. of nitrogen. Albumin, grade 2, was usually present. Microscopic examination of the sediment revealed numerous small hyaline casts and some erythrocytes.

These analyses indicate that a substantial increase in the intake of nitrogen resulted in a substantial increase in the output of nitrogen and in a slight increase in diuresis.

Measurement of renal corpuscles and convoluted tubules revealed a marked increase in the average (of ten) diameter in the hypertrophied kidney over the diameter in the kidney removed at operation. A comparison of the amounts of increase under experimental (high protein) and control conditions (table 9) indicated that the larger kidneys in experimental animals have larger corpuscles and convoluted tubules. The tubules probably also increase in length as well as in diameter. It is clear that enlargement of the kidney must be due to an increase in the size of the corpuscles and tubules (figs. 3 and 4).

In addition to the hypertrophic changes, inflammatory reactions in the renal parenchyma were sometimes observed, particularly in the more prolonged experiments. These reactions corresponded to those of spontaneous nephritis in the rat, which is a form of exudative interstitial nephritis. Striking features (fig. 5) of this lesion are lymphocytic infiltration which causes atrophy from disuse of the tubules secondary to pressure. The affected tubules are dilated, and the epithelium is thinned. The use of special stains, such as azocarmine, on the basement membrane did not disclose the thickening which characterizes some forms of nephropathy in man.

It is fair to conclude, therefore, that the high protein diet stimulated compensatory renal hypertrophy in albino rats. The nephritis was probably only indirectly related to the diet, because in a prolonged experiment in which the diet is somewhat abnormal infections appear more likely to occur.

Experiment 2A. Effect of Diet Containing Urea Fed for Eight Weeks.—After several trials it was found that rats would eat a mixture of the standard ration and crystalline urea in the proportion of 4 to 1 (20 per cent urea) by weight. The animals were somewhat retarded in general bodily growth. Nevertheless, apparently significant results were obtained.

The experimental animals of the litter, including one animal which was not operated on, were fed the mixture of the standard ration and urea beginning on the day following operation. The control animals of the litter, including the second animal which was not operated on,

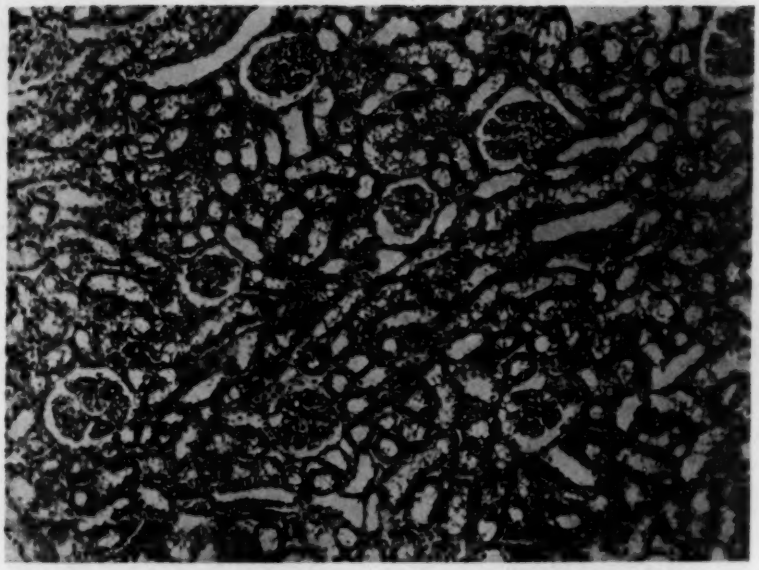

Fig. 3 (experiment 1B, series 1, rat 64).—Photomicrograph of a section from the right kidney, removed at operation when the rat was 62 days old, showing normal corpuscles and tubules (× 100).

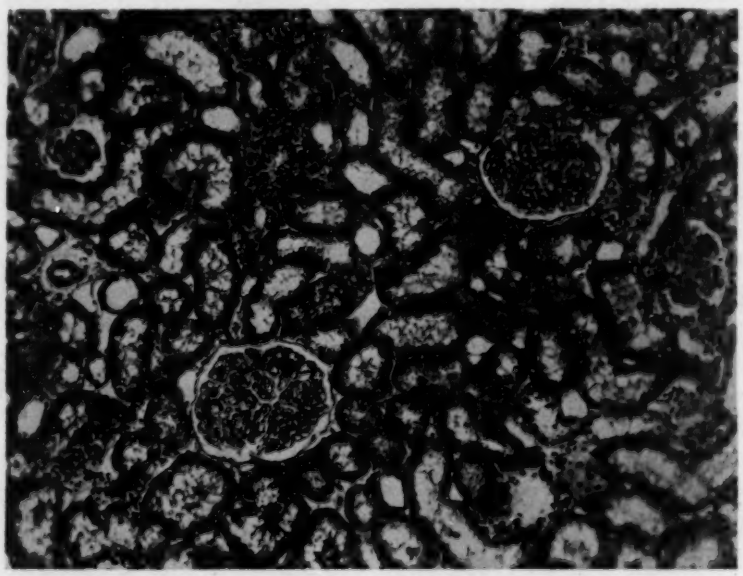

Fig. 4 (experiment 1B, series 1, rat 64).—Photomicrograph of a section from the left kidney removed at necropsy, after one hundred and eighty-eight days on a high protein diet, showing marked hypertrophy of the corpuscles and tubules (× 100).

were fed only the standard ration. The experiment was terminated after eight weeks.

Results (fig. 6, tables 9, 10 and 11): The effect of this diet was also to increase the rate of hypertrophy of the remaining kidney (fig. 6). The average increase in weight of the hypertrophied kidneys in the experimental animals was 141 per cent, whereas for the control animals the figure was 93.6 per cent (table 10). The average percentage of increase in body weight was enormous, averaging 11.1 as compared with 6.81 for the control group. The index number for the animal not operated on, 13.6, suggests that the ingestion of urea stimulated bilateral hypertrophy also, because on the control diet a similar animal of the same litter, not operated on, had an index number of only 8.98.

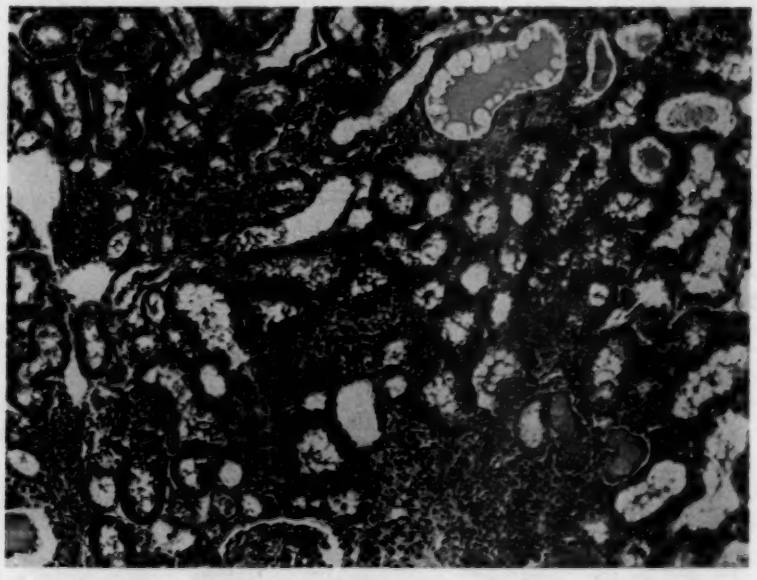

Fig. 5 (experiment 1B, series 1, rat 64).—Photomicrograph of a section from the left kidney (same as fig. 2), showing an area of exudative interstitial nephritis with dilated atrophic tubules (× 100).

Experiment 2B. Effect of a Diet Containing Urea Fed for Six Months.—The same mixture on the standard ration and urea was used as in experiment 2A. This experiment was controlled with the same group of animals that was employed for experiment 1B, and estimations of the total intake of nitrogen and water, urinalysis and microscopic study of the kidneys also were carried out.

Results (fig. 2; tables 3, 5, 6, 8, 9 and 11): In this experiment also the diet containing urea increased the hypertrophy of the remaining kidney, as is shown graphically in figure 2. The curve ascends sharply at first, paralleling the similar curve of experiment 1B and finally crossing it to level off near index number 12 after about one hundred days

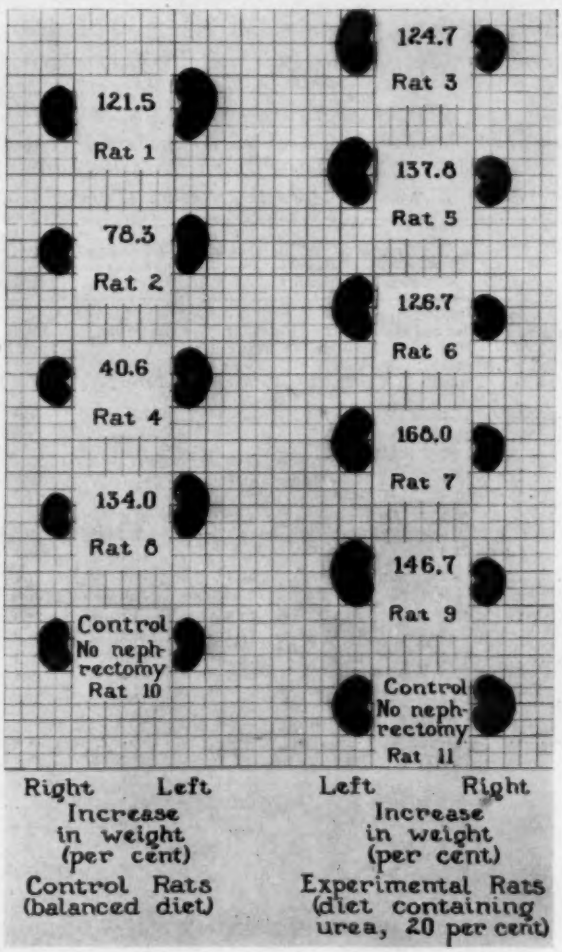

Fig. 6 (experiment 2A, series 1).—Kidneys from albino rats showing the effect of a diet containing 20 per cent of urea fed for eight weeks after excision of the right kidney.

of the experiment. The character of the curve clearly suggests that urea stimulates more hypertrophy than was stimulated by a high protein diet, at least in the middle phase (from one hundred and ten to two hundred and twenty days) of the experiment. This is due to the

fact that animals on a diet containing urea gain weight slowly and are always somewhat stunted; the kidney, on the other hand, grows rapidly; the consequence is a larger amount of renal tissue per unit of body weight. It should be noted, however, that after one hundred and eighty-eight days on a high protein diet one rat surpassed all the rats fed a diet containing urea, having an index number of 14.36.

Quantitative chemical studies (table 8) revealed that the average twenty-four hour intake of nitrogen was 1,668.35 mg. The average

TABLE 9.—*Comparison of Percentage of Increase in Mean Values of Diameters of Glomeruli and Tubules of Animals on a Standard Diet and on an Experimental (High Protein Diet and Diet Containing Urea) Diet in Experiments 1B and 2B, Series 1*

	Percentage of Increase in Mean Values of Diameters of					
	Renal Corpuscles			Tubules		
Age When Killed (Ten Day Intervals)	Standard Balanced Diet (Control; Table 2)	High Protein Diet (Table 2)	Diet Containing Urea (Table 3)	Standard Balanced Diet (Control; Table 2)	High Protein Diet (Table 2)	Diet Containing Urea (Table 3)
60 to 69	....	—4.8	....	....	7.6	
70 to 79	17.1	23.7	....	23.9	19.9	
	7.2			11.9		
80 to 89	....	31.1	....	....	20.8	
		33.9			42.9	
90 to 99	20.1	....	....	27.3		
	7.9	26.1	14.8	7.3	30.7	4.4
	14.0			22.8		
100 to 109	7.7	23.5	31.6	10.6	17.7	24.2
	5.6			21.4		
110 to 119	13.1	43.9	16.5	8.1	38.0	26.7
	29.5	38.5	17.3	8.2	30.9	16.6
130 to 139	17.1	37.6	....	17.3	29.4	
	17.9	19.8	43.6	36.5	19.4	34.0
140 to 149	20.0	43.1	....	18.3	31.9	
	20.9			12.3		
150 to 159	....	....	80.8	....	....	32.1
170 to 179	33.9	69.2	....	28.7	35.0	
	35.1			22.7		
210 to 219	31.9	....	68.8	22.5	....	40.3
250 to 259	40.1	39.9	....	6.0	54.9	
	46.7			14.9		

intake of water was 56.2 cc., and the average output of urine was 43 cc., which represents marked diuresis. The urine contained 973.8 mg. of nitrogen. Albuminuria, graded 1 to 2, with a few hyaline casts and erythrocytes, was noted.

These analyses indicate that a substantial increase in the intake of nitrogen in the form of urea resulted in a substantial increase in the output of nitrogen.

As in the experiment with the high protein diet, measurements of renal corpuscles and convoluted tubules (table 9) showed significant

differences between control and experimental observations, indicating that the enlargement was due to an increase in size of these components.

Inflammatory reactions in the renal parenchyma were less marked than in the previous experiment but were of the same general character.

TABLE 10.—*Comparison of Renal Weight and Body Weight of Animals in Experiment 2A, Series 1*

	Rat Number	Rat Number in Series*	Sex	Increase in Body Weight		Increase in Renal Weight		Relation of Renal Weight to Body Weight (Index Number)
				Grams	Percentage	Grams	Percentage	
Control portion of litter; balanced diet	70	1	M	8.0	7.1	0.70	121.5	5.81
	71	2	F	34.1	36.9	0.36	78.3	6.48
	72	4	F	37.0	39.6	0.27	40.6	7.16
	73	8	M	76.0	102.7	0.67	134.0	7.80
	Means			38.8	46.1	0.50	93.6	6.81
	Ranges			8.0 to 76.0	7.1 to 102.7	0.27 to 0.70	40.6 to 134.0	5.81 to 7.80
Experimental portion of litter; diet containing urea	74	10	F	38.5	33.8	(Control, no operation)		8.98
	75	11	M	66.5	58.3	(Control, no operation)		13.60
	76	3	F	16.3	17.0	0.68	124.7	10.93
	77	5	M	46.5	41.4	0.98	137.8	10.86
	78	6	M	30.0	33.1	0.71	126.7	10.54
	79	7	F	16.5	16.5	0.84	168.0	11.50
	80	9	F	33.5	36.0	0.88	146.7	11.69
	Means			28.6	28.8	0.82	141.0	11.10
	Ranges			16.3 to 46.5	16.5 to 41.4	0.68 to 0.98	124.7 to 168.0	10.54 to 11.69

* These numbers appear in figure 6.

TABLE 11.—*Comparison of Renal Weight and Body Weight of Experimental Animals (on Diet Containing Urea) in Experiment 2 B, Series 1*

Rat Number	Sex	Age When Killed, Days	Body Weight, Kg.	Renal Weight, Gm.	Relation of Renal Weight to Body Weight (Index Number)
81	M	82	0.1370	0.93	6.79
82	F	96	0.1180	0.95	8.05
83	F	103	0.1010	1.34	13.27
67	F	110	0.1580	1.00	6.36
69	M	117	0.1970	1.74	8.86
84	F	138	0.1220	1.26	10.37
66	M	159	0.1625	1.96	12.09
68	F	220	0.1590	1.87	11.79

It may be concluded, therefore, that a diet containing crystalline urea in a concentration of 20 per cent stimulated compensatory renal hypertrophy in albino rats.

SERIES 2. EFFECT OF INCREASING THE FLOW OF URINE ON COMPENSATORY HYPERTROPHY OF THE KIDNEYS OF RATS

The largest constituent of urine is water. It was desired to determine whether increasing the water excreted by the remaining kidney would increase the rate of compensatory hypertrophy. It was recog-

nized that any agent which might be used to produce a greater excretion of urine might also cause a change in the rate of hypertrophy per se.

Experiment 1. Effect of Intraperitoneal Infusion of a Solution of Sucrose.—Sucrose, after being absorbed from the peritoneal cavity into the blood stream, is excreted by the kidneys and produces an increased flow of urine. Twenty cubic centimeters of a 20 per cent solution of sucrose was introduced into the peritoneal cavity of each animal of the experimental group three times weekly; a sterile syringe and needle were used. The increased flow of urine was disappointingly small. Twenty cubic centimeters of physiologic solution of sodium chloride was injected into each of the animals of the control group at similar intervals and in a similar fashion. The animals were maintained on the standard diet. The experiments were terminated at the end of the eighth week.

Results: The hypertrophied kidneys of the animals in the experimental group appeared to be slightly larger than those of animals in the control group. The average increase in weight of the hypertrophied kidney was 151.4 per cent as compared with 108.3 per cent for the control group. However, the animals which received the injections of sucrose gained more body weight than the control animals, that is, 78.5 per cent, as compared with 68.3 per cent. In spite of this difference the amount of renal tissue per unit of body weight was larger (index number 8.71) than the amount in the control group (7.81). This result, although inconclusive, is at least suggestive.

Microscopic examination of the kidney failed to reveal any changes other than those of hypertrophy. Inflammatory or degenerative changes were not evident.

Experiment 2. Effect of a Diet Containing a Large Quantity of Sodium Chloride.—After several trials it was found that a diet containing sodium chloride in the proportion of 15 per cent by weight was tolerated by rats and that such a mixture stimulated the drinking of water. By this method the intake of water, and consequently the output of urine, were greatly augmented.

Results: The urinary output averaged 82.4 cc. in twenty-four hours for four animals, truly a pronounced diuresis. In some instances the amount of urine excreted in twenty-four hours exceeded in weight the body weight of the rat. The urine, however, contained little solid matter, and on the average only 233.20 mg. of nitrogen was contained in a twenty-four hours specimen. No albumin or foreign elements were present.

The hypertrophy was no greater under experimental than under control conditions (fig. 7). The average increase in the experimental animals was 103.4 per cent; in the control animals, 122.4 per cent. The increase in body weight was considerably retarded, amounting to 44.8

per cent as compared with 87.3 per cent for the control group, which accounts for the fact that the amount of renal tissue per unit of body weight was greater (index number 9.34) under the experimental conditions than under control conditions (index number 7.14).

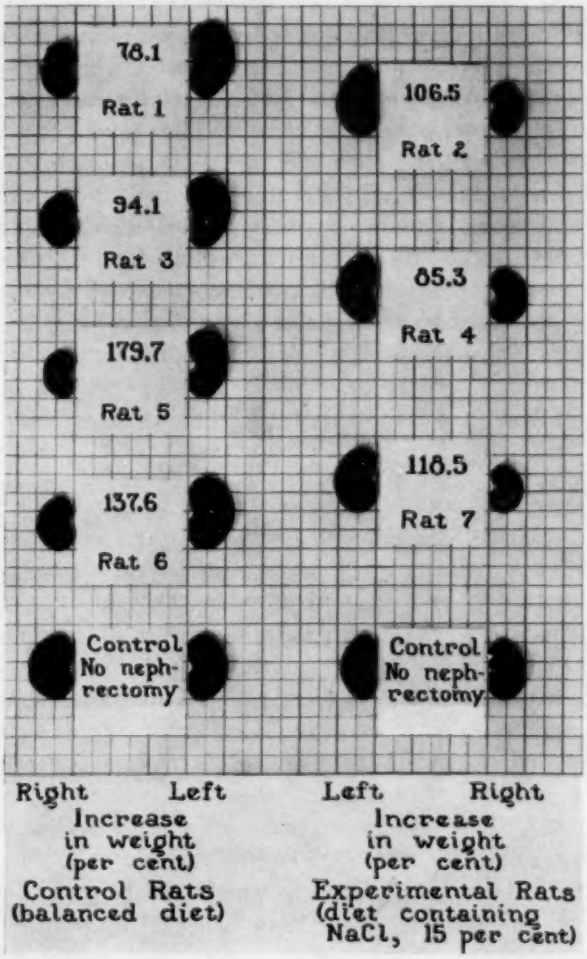

Fig. 7 (experiment 2, series 2).—Kidneys from albino rats showing the effect of a diet containing 15 per cent of sodium chloride fed for eight weeks after excision of the right kidney.

SERIES 3. EFFECT OF FORCED EXERCISE ON COMPENSATORY HYPERTROPHY OF THE KIDNEYS OF RATS

It is generally known that exercise increases metabolism. With this fact in mind it was decided to have unilaterally nephrectomized rats undergo forced exercise to observe its effect on the kidney during com-

pensatory hypertrophy. The exercise drums were turned by an electric motor at a rate which kept the rats walking continuously in order to keep upright. The rats walked a computed distance of 16,400 feet (4,998 meters) in eleven hours. Since the rat is to a certain extent a nocturnal animal, the exercising was begun at 6:00 p. m. and continued till 6:00 a. m., with an hour at midnight and one night a week for rest. Slight signs of fatigue usually developed toward morning. In the control animals the exercise was limited to normal activities possible in the cage. The duration of this experiment was nine weeks.

Results: A comparison of renal weights and body weights of animals in the control and experimental groups shows that exercise did not stimulate hypertrophy. In fact, the contrary appears to be the case because the average increase in renal weight was only 79.8 per cent as compared with 135.7 per cent for the control animals. This, however, is apparently due to the fact that in the exercised animals the body weight did not increase to the same degree as in the control animals, which accounts also for the fact that the index numbers are approximately the same.

SERIES 4. EFFECT OF PREGNANCY ON COMPENSATORY HYPERTROPHY OF KIDNEYS OF RATS

Pregnancy is characterized by profound changes in the metabolism of several organs. Women with diseased kidneys are said to be subjected to greater hazards by gestation than normal women, which suggests that possibly a greater burden on the renal function accompanies the pregnant state. For this reason the effect of pregnancy on the remaining kidney of unilaterally nephrectomized rats was studied.

Five females of a litter were used for this purpose. One rat failed to survive nephrectomy. Two of the remaining four animals were mated with potent males. During the ensuing six months one rat bore three litters totaling twenty-five rats, and the other bore four litters totaling thirty-two rats. The control rats were not mated. While the series is small, changes at all significant should be evident.

Results: The increase in body weight in the control and in the experimental animals was of the same order of magnitude, but in the control group the hypertrophy of the kidney was slightly greater, 136.9 per cent, than in the experimental group, 126.8 per cent. This difference is reflected in the index numbers, which show that on an average the control animals exhibited somewhat more renal tissue per unit of body weight (8.6) than did the experimental animals (7.76).

Microscopic examination of sections of the kidney revealed only the changes of hypertrophy; no inflammatory or degenerative lesions were detected.

SERIES 5. EFFECT OF RENAL DENERVATION, DECAPSULATION AND BANDING OF THE RENAL ARTERY ON COMPENSATORY HYPERTROPHY OF THE KIDNEYS OF RABBITS

As previously stated, rabbits were found to be inferior to rats as experimental animals for the study of compensatory hypertrophy of the kidney. However, owing to the nature of some of the experiments which we desired to perform, rabbits were chosen because of their larger size.

Experiment 1. Effect of Denervation of the Remaining Kidney.—The exact relation of renal nerves to renal function is not known. Recent work by Milles, Müller and Peterson [2] and Caldwell [3] and others suggests that the nerve supply is chiefly concerned with the vascular system. Indirectly the renal function may be affected by alterations in the vascular bed secondary to interference with the nerve supply. Caldwell's work showed that a denervated kidney excreted more urine than its nondenervated mate, a condition which persisted for some months. In general, such studies suggest that the nerves indirectly tend to inhibit renal secretion. Thus, a denervated remaining kidney, its mate having been removed, might hypertrophy with greater rapidity than a kidney with the nerves intact.

The idea was tested by dividing each of the litters of rabbits equally for control and experimental purposes. The right kidney was removed, and the left kidney was denervated. The technic of denervation consisted in stripping all fat and connective tissue from the renal artery or arteries, which are often multiple in rabbits, and from the vein or veins. The adventitia of the renal artery was then teased loose with a small thumb forceps and was stripped from a considerable section of the artery throughout its circumference. Most of the nerve fibers in the renal pedicle were sectioned by this technic.

The experiment with one litter was terminated one hundred and ninety-five days, and the other one hundred and one days, after operation.

Results (table 12): One experimental and three control animals of litter 1 survived. The denervated kidney increased 132.17 per cent by weight, while the average increase for the control animals was 78.08 per cent. The relative increase in body weight was approximately the same. A comparison of the index numbers shows that the amounts of renal tissue per unit of body weight were about the same.

One control and two experimental animals of litter 2 survived. Again the denervated kidneys of these rabbits were considerably larger (102.4 per cent increase) than those of the control animals (64.45 per cent

2. Milles, G.; Müller, E. F., and Peterson, W. F.: Proc. Soc. Exper. Biol. & Med. **28**:354, 1931.

3. Caldwell, J. M., Jr.: J. Urol. **28**:323, 1932.

increase), and in this litter the index number for the experimental animals was 4.62, a figure significantly larger than that for the control animals, 3.64.

Experiment 2. Effect of Renal Decapsulation.—The renal capsule consists chiefly of fibrous connective tissue with but small amounts of

TABLE 12.—*Comparison of Renal Weight and Body Weight of Animals in Experiment 1, Series 5*

Rat Number	Sex	Increase in Body Weight		Increase in Renal Weight		Relation of Renal Weight to Body Weight (Index Number)
		Grams	Percentage	Grams	Percentage	
		Control Animals (Litter 1)				
85	M	0.985	72.69	4.58	80.51	4.39
86	M	0.855	74.67	3.66	80.86	4.87
87	F	1.145	90.52	4.60	72.86	4.48
Means.....		0.995	79.29	4.28	78.08	4.58
		Experimental Animal				
88	F	1.063	80.71	5.86	132.17	4.32
		Control Animal (Litter 2)				
89	M	0.748	39.47	3.78	64.45	3.64
		Experimental Animals				
90	F	0.760	35.51	4.74	69.59	3.98
91	F	0.526	25.53	5.64	70.76	5.26
Means.....		0.643	30.52	5.19	102.40	4.62

TABLE 13.—*Comparison of Renal Weight and Body Weight of Animals in Experiment 2, Series 5*

Rat Number	Sex	Increase in Body Weight		Increase in Renal Weight		Relation of Renal Weight to Body Weight (Index Number)
		Grams	Percentage	Grams	Percentage	
		Control Animals				
92	F	0.725	44.82	2.80	43.83	3.84
93	M	1.200	74.07	3.14	47.68	3.44
Means.....		0.962	59.44	2.96	45.76	3.64
		Experimental Animals				
94	F	1.150	70.98	3.61	58.46	3.53
95	M	1.055	67.83	7.38	107.03	5.47
Means.....		1.102	69.40	5.50	82.74	4.50

elastic connective tissue and smooth muscle. In consequence the capsule is relatively inelastic. The thought that the capsule might retard the hypertrophic process by compressing the expanding parenchyma prompted this experiment.

A litter of four rabbits was divided equally, and the pair selected for experimental purposes, in addition to excision of the right kidney, were subjected to decapsulation of the left kidney. Decapsulation was accomplished simply by slitting the capsule and then stripping it from

the parenchyma and cutting it free at the hilus. The animals were killed after six months.

Results (table 13): The decapsulated kidneys were substantially larger than the kidneys of the control animals, the average increase being 82.74 per cent as compared with 45.76 per cent. This advantage is independent of differences in body weight, for the index number representing the amount of renal tissue per unit of body weight, 4.5, is significantly larger than the index number, 3.64, for the control animals.

SUMMARY

The right kidney was removed from rats and rabbits. Proper control observations were made for each series of experiments. Since the investigation included several separate series of experiments it can best be summarized by presenting the results which were conclusive separately from those which were inconclusive.

Conclusive Results.—In animals that were fed a diet rich in protein much larger kidneys developed than in the control animals on the standard diet. Evidence indicates that the enlargement was due to an increase in the size of the renal corpuscles and tubules.

In animals that were fed a diet containing 20 per cent urea much larger kidneys developed than in the control animals on the standard diet. Evidence indicates that the enlargement was due to an increase in the size of renal corpuscles and tubules.

In animals that were fed on a diet containing a 15 per cent concentration of sodium chloride the kidneys secreted a much larger amount of urine but did not grow larger than in the control animals.

In animals that were placed on forced exercise the kidneys did not show more hypertrophy than in the control animals.

Animals that were permitted to undergo gestation several times did not have larger kidneys than the control animals.

Inconclusive or Suggestive Results.—The intraperitoneal injection of a 20 per cent solution of sucrose caused a slight increase in the amount of urine secreted, and slightly larger kidneys were noted than in the control animals which received intraperitoneal injections of physiologic solution of sodium chloride. In animals in which the remaining kidney was denervated the kidneys tended to grow slightly larger than in the control animals in which the nerves were left intact.

In animals in which the remaining kidney was decapsulated there developed kidneys slightly larger than in the control animals in which the capsule was left intact.

EXPERIMENTAL GOITER

FUNCTIONAL, CHEMICAL AND HISTOLOGIC STUDIES

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In previous communications,[1] the production of goiter in white rats was reported. It was found that a deficiency of iodine by itself is not the essential cause of goiter and that there must be a positive goitrogenic factor present. Calcium chloride proved to be very effective in producing enlarged thyroid glands in white rats. The structure of these experimental goiters varied according to the amount of iodine which was contained in the goiter-producing diet. An excess of calcium and at the same time a low content of iodine in the food caused parenchymatous goiter with very scanty colloid, while the same calcium-rich diet with a relatively high amount of iodine produced colloid goiter. By these experiments it was demonstrated for the first time that colloid goiter is not, as most students of goiter believe, an involutionary stage of hyperplastic goiter, but a special form of endemic goiter which may and does develop primarily from a normal thyroid gland.

Comparative studies [2] on European and North American material have shown that colloid goiter is the prevailing form of endemic goiter in level countries where hyperthyroidism is common, while parenchymatous, colloid-poor goiter is the prototype in mountainous goitrous regions and is often associated with signs of deficient thyroid function.

The problem therefore presented itself whether the experimental goiters in white rats fed with a diet rich in calcium but varying in iodine differed not only in structure but also in function.

METHOD

The studies on which this paper is based were made on three groups of white rats which were of about the same age and weight but had received different food for a period of one hundred and forty days. Six male rats were used as controls. They were fed on the stock diet, which was well balanced and contained green vegetables, meat and cereals. The drinking water was Wichita city

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From the Department of Pathology, St. Francis Hospital.

Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1. Hellwig, C. A.: Arch. Path. **11**:709, 1931; West. J. Surg. **41**:453, 1933; Endocrinol. **18**:197, 1934.

2. Hellwig, C. A.: Surg., Gynec. & Obst. **55**:35, 1932.

water. The other two groups received a basal diet consisting of cornmeal (50 per cent) and rolled oats (50 per cent) boiled in distilled water. The first of these experimental series had 2 per cent calcium chloride added to the basal diet, so that each rat's daily intake was about 0.2 Gm. of calcium chloride. While, in this calcium group, there were six male animals about 1 year old, the second experimental lot consisted of six males and four females of the same age. Males and females were kept in separate Dormer cages. The second group was fed the same calcium-rich diet as the first, but in addition each animal was given daily 2 micrograms of potassium iodide. The histologic examination which followed the functional tests showed that the resulting goiters in my new series were identical with the thyroid changes obtained in my previous studies. The calcium-rich diet without iodine produced parenchymatous goiters, while the calcium-rich and iodine-rich diet caused colloid goiters.

EXPERIMENTAL RESULTS

Since it did not seem practical to determine the basal metabolism on such small animals, I used as a measure of thyroid function the sensitivity of the white rats to lack of oxygen. Streuli [3] had shown that rats, after thyroidectomy, are much more resistant to low oxygen content of the air than are normal animals. Asher and Wagner,[4] on the other hand, demonstrated that in white rats feeding of thyroid substance will increase the sensitivity to oxygen deficiency. From these experiments, the conclusion can be drawn that the sensitivity to lack of oxygen depends on the activity of the thyroid gland.

Figure 1 illustrates the apparatus which was used in my functional studies. It was set up according to the directions given by de Quervain.[5] A large vacuum bell jar was placed on a polished marble plate and the contact made air-tight by thick greasing. A mercury manometer and a water suction pump were connected with the bell jar by heavy rubber tubing. Under the bell jar was a dish with 3 per cent potassium hydroxide for absorbing the carbon dioxide of the expired air. The experimental animal was placed on top of a piece of mesh wire covering the dish containing potassium hydroxide. By slow suction, the air pressure in the bell jar was lowered gradually until the manometer indicated 160 mm. of pressure. This point was reached in about twenty minutes. At the end of the experiment, fresh air was admitted through a valve.

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3. Streuli, H.: Biochem. Ztschr. **87**:359, 1918; cited by de Quervain, F., and Abelin, I., in Abderhalden, E.: Handbuch der biologischen Arbeitsmethoden, Berlin, Urban & Schwarzenberg, 1932, vol. 8, p. 1612.

4. Asher, L., and Wagner, H.: Ztschr. f. d. ges. exper. Med. **68**:32, 1929.

5. de Quervain, F.: Die experimentelle Prüfung der Schilddrüsenfunktion, in Abderhalden, E.: Handbuch der biologischen Arbeitsmethoden, Berlin, Urban & Schwarzenberg, 1932, vol. 8, p. 1613.

During the experiment, most animals presented a definite sequence of symptoms. After being placed under the bell jar, they overcame their first surprise very rapidly and were sitting up in a natural position, washing themselves carefully. When the oxygen pressure decreased, the rats stopped all movements, lay down flat and kept the head as low as possible. The respiration at this first stage of dyspnea was rapid and superficial. The second, much more serious stage was characterized by restlessness. Apparently the animals tried to escape from the dangerous place. They searched eagerly around, stood up on the wall of the bell jar and finally jumped repeatedly against the glass top.

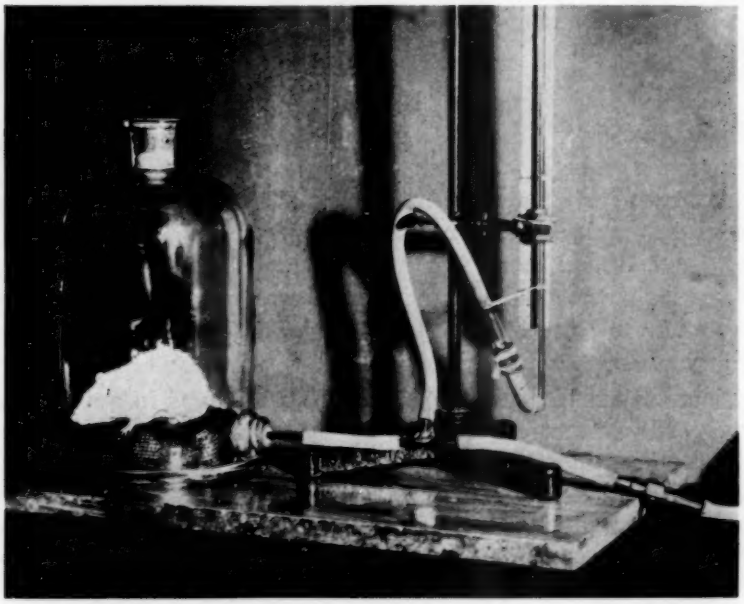

Fig. 1.—Experimental set-up for Asher's method of determining thyroid function.

The breathing became very difficult, infrequent and deep, the accessory muscles being used. In the final stage, partial paralysis of the extremities was noticed; the animals crawled aimlessly around and finally fell to one side, losing consciousness. The mouth was wide open, and respiration was of the Cheyne-Stokes type. Death was imminent and could be prevented only by admittance of fresh air.

A detailed protocol was dictated during each experiment, and the changes in breathing and in general behavior of the animals were recorded at the different manometer readings. The reaction of all animals in each particular series was the same. There was an appreciable difference in the sensitivity to low oxygen pressure between the two

series of white rats which had received the same calcium-rich diet, but which differed in their intake of iodine. The first serious symptoms of air hunger were noticeable in the animals fed a calcium-rich, iodine-poor diet at 330 mm. of mercury, while the rats on a calcium-rich, iodine-rich diet presented the same symptoms at 429.3 mm. The control animals reacted in the same manner when the air pressure decreased to 390 mm. Striking differences were noted also in the duration of unconsciousness after fresh air was admitted. While the calcium-fed animals recovered within a few minutes from the severe symptoms of

Fig. 2.—A papillary adenoma in the left lobe of the thyroid gland, produced by a calcium-rich, iodine-poor diet. *T* indicates trachea; *O*, the esophagus.

the third stage, only 40 per cent of the rats fed the calcium-rich, iodine-rich diet were able to walk when they were taken from the bell jar. Even after hours, they still presented severe symptoms of dyspnea.

Several days after these functional studies, all white rats were anesthetized with chloroform and killed by bleeding from the heart. The thyroid glands were removed immediately after death, weighed and measured and a small portion of each gland was saved for histologic study. The glands of all rats on the same diet were pooled, and the iodine content of the composite and weighed glands was then deter-

mined by the Harington method. The blood was collected from the rats of each series and the serum calcium determined.

In the controls, the average weight of the whole gland was 26.3 mg.; the average size of the right lobe was 5.1 by 1.7 by 3.5 mm. and that of the left, 4.9 by 1.9 by 3.1 mm. The group which was fed a calcium-rich and iodine-poor diet presented marked enlargement of the thyroid gland. The right lobe measured 10.5 by 4.5 by 5 mm., the left 9.5 by 3.2 by 4.5 mm., and the average weight was 73.8 mg. Two of the thyroid glands in the latter group had a large adenoma. The thyroid

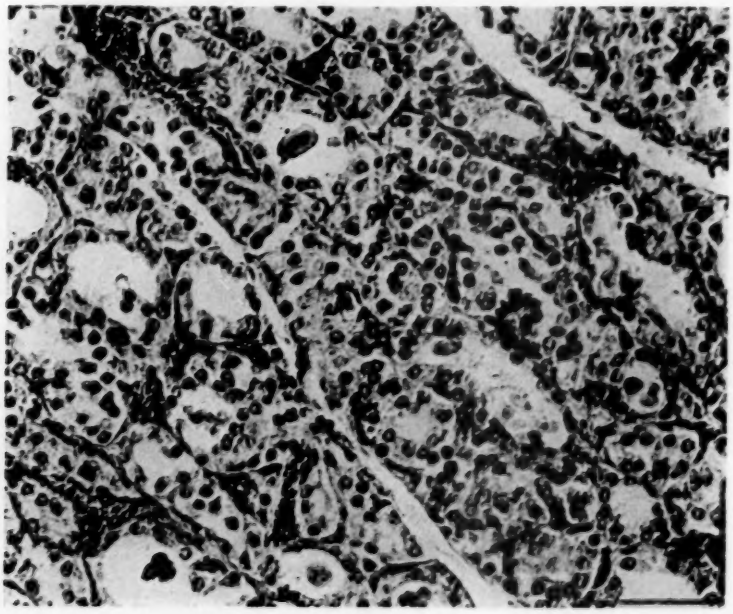

Fig. 3.—Parenchymatous goiter developing after a diet rich in calcium and poor in iodine.

glands of the animals receiving a calcium-rich and iodine-rich diet were also definitely enlarged; the average weight was 40.9 mg.; the right lobe measured 8.4 by 2.4 by 4.1 mm., and the left, 8.3 by 2.1 by 3.5 mm.

The results of the chemical analyses of the thyroid glands coincided with the histologic changes. The parenchymatous, colloid-poor glands from the series receiving calcium-rich and iodine-poor food were lowest in iodine concentration, i. e., 0.0045 per cent (on the basis of fresh tissues). These glands contained approximately only one third as much iodine as glands from animals fed the stock diet. When compared with the values for glands from animals which were given the

calcium-rich, iodine-rich diet, these values became approximately one tenth, since the concentration of iodine in the third series was 0.0405 per cent. Histologically the iodine-rich glands presented the picture of a proliferant colloid goiter.

The serum calcium, which was determined by the method of Clarke, differed widely between the two experimental groups. In the series with calcium-rich, iodine-poor diet the calcium content was 12 mg. per hundred cubic centimeters of blood, while the animals receiving the calcium-rich, iodine-rich food, had only 6.6 mg.

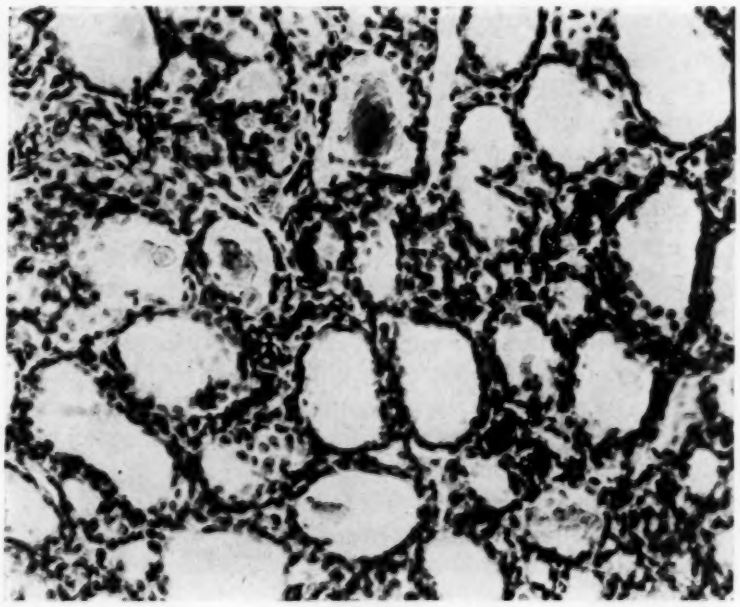

Fig. 4.—Colloid goiter developing after a diet rich in calcium and iodine.

COMMENT

Judging from the literature, there are only very few functional studies made on experimental goiter. Levine, Remington and Von Kolnitz [6] reported that the basal metabolism in four white rats with experimental hyperplastic goiter was 23 per cent below normal. The results in my first series are in accord with these findings. The six rats in which colloid-poor, parenchymatous goiter was produced by a calcium-rich and iodine-poor diet were much more resistant to lack

6. Levine, H.; Remington, R. E., and von Kolnitz, H.: J. Nutrition **6**:325, 1933.

of oxygen than were normal animals; i. e., they showed a low thyroid function.

Functional studies on primary colloid goiter produced in experimental animals have never been made so far. I was able to produce colloid goiter by feeding white rats with a calcium-rich and iodine-rich diet. These goiters were functionally much more active than the parenchymatous, colloid-poor goiters of the first experimental series. The animals with colloid goiters tolerated lack of oxygen much less than did normal animals or the ones with parenchymatous goiters. My observations can be compared somewhat with metabolic studies made by Webster [7] on rabbits. However, in his experiments the colloid goiter was secondary to hyperplastic, colloid-poor goiter. By feeding cabbage, Webster produced first hyperplastic goiters; then he gave relatively large intraperitoneal doses of potassium iodide and thus changed the parenchymatous goiters into colloid goiters. The latter were found to be associated with a marked increase in metabolic rate. In my experiments, colloid goiters were produced directly from normal glands by a diet which contained a strong goitrogenic factor and, at the same time, a relatively high amount of iodine. These experimental colloid goiters represent, according to my functional studies, a more active stage of thyroid function than the parenchymatous goiters produced by a calcium-rich and iodine-poor diet. The former resemble the colloid goiters of the lowlands, where different forms of hyperthyroidism are common, while the parenchymatous goiters with low function can be compared with the typical endemic goiter of the mountains, where hypothyroidism is frequently found.

While the determinations of iodine in my experimental goiters only confirmed the histologic observations, the study of the serum calcium in the animals presented interesting data. The blood of the animals with parenchymatous goiter contained twice the amount of calcium found in the blood of the white rats with colloid goiter. Baumann, Kurland and Metzger [8] reported that in goiter produced in the rabbit by cabbage feeding there is a definite retention of calcium in the body. They believe that the retention of calcium is directly associated with the development of simple goiter. Jansen,[9] studying the blood calcium in various abnormal conditions in human beings, found that in hyperthyroidism the blood calcium is low and in hypothyroidism high. My calcium studies in the two experimental series are completely in accord with these reports.

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7. Webster, B.: Bull. Johns Hopkins Hosp. **45**:215, 1929.

8. Baumann, E. J.; Kurland, S., and Metzger, N.: J. Biol. Chem. **94**:383, 1931.

9. Jansen, W. H.: Deutsches Arch. f. klin. Med. **143**:14, 1922.

SUMMARY

Calcium-rich diet produces definite goiters in white rats. When the goitrogenic diet is very poor in iodine, the goiters are parenchymatous, colloid-poor and iodine-poor. When the goitrogenic diet is relatively rich in iodine, the goiters produced are colloid-rich and iodine-rich. Not only the structure of the experimental goiters, but also the function, varies according to the iodine content of the goitrogenic diet.

The experimental colloid goiters show a higher thyroid function than the parenchymatous, colloid-poor goiters.

The calcium content of the blood serum is twice as high in the animals with parenchymatous goiters as it is in the animals with colloid goiters.

The results of these functional, chemical and morphologic studies are in accord with the epidemiological and clinical facts on human goiter.

Case Reports

RHABDOMYOMATOSIS OF THE HEART IN A NEGRO

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Interest in the relatively rare tumors of the striated muscle tissue has been revived in recent years by the investigations of Abrikosoff,[1] Glasunow,[2] Wolbach [3] and others, who have described a new and heretofore unknown type of tumor occurring in muscle tissue, the myoblastic myoma, proposed a classification of these neoplasms based on their degree of differentiation and added to the knowledge of the histogenesis of the myofibrils in the cells of rhabdomyomatous blastomas. In this group of neoplasms the congenital blastomatoid formations of striated muscle found in the heart take a special position for various reasons.

They are extremely rare. Farber,[4] who was the last author to report on this subject, collected reports of forty-three cases of rhabdomyomas of the heart from the literature; only three of these cases occurred in this country. These tumors are usually found in infants or young children and occur only rarely in adults. The oldest person in whom the formations were observed was 37 years of age (Steinbiss [5]). The neoplasms have generally been observed in connection with other congenital formative disturbances, such as: tuberous sclerosis and multiple gliomas of the brain; angiosarcoma, angiolipoma, adenoma, hypernephroma, simple cyst, embryonic rests without glomeruli of the kidney; multiple sebaceous gland adenomas of Pringle of the skin; embryonic malformation of the pancreas; rhabdomyolipoma of the heart, etc. It was noted that in some cases the parenchymatous organs (liver, spleen, kidney and heart) were markedly enlarged (Farber), reaching twice the weight of the corresponding normal organs. Congenital rhabdomyomas of the heart represent, therefore, apparently a partial manifestation of a generalized developmental disturbance often affecting various organic systems.

On account of the frequent coexistence of rhabdomyomatosis of the heart and tuberous sclerosis (according to Bundschuh [6] the condition is encountered in 50 per cent of the cases of tuberous sclerosis),

From the Department of Pathology and Graduate Hospital of the University of Pennsylvania.

1. Abrikosoff, A. J.: Beitr. z. path. Anat. u. z. allg. Path. **45**:376, 1909; Virchows Arch. f. path. Anat **260**:215, 1926

2. Glasunow, M.: Frankfurt. Ztschr. f. Path. **45**:328, 1933.

3. Wolbach, S. B.: Anat. Rec. **37**:255, 1928.

4. Farber, S.: Am. J. Path. **7**:105, 1931.

5. Steinbiss, W.: Virchows Arch. f. path. Anat. **243**:22, 1923.

6. Bundschuh, E.: Beitr. z. path. Anat. u. z. allg. Path. **54**:278, 1912.

which results in imbecility and epileptoid attacks, rhabdomyomatosis is most frequently observed among the inmates of insane asylums and is therefore rarely seen by pathologists in general hospitals. Steinbiss reported six cases of rhabdomyomas of the heart observed in twenty-one cases of tuberous sclerosis which occurred in one institution of this type. All the cases so far recorded have occurred in members of the white race.

Farber has classified rhabdomyomas of the heart into three groups: (1) solitary tumors (eight cases), (2) multiple tumors (thirty-four cases) and (3) diffuse rhabdomyomatosis (one case). The last figure is apparently incorrect, as diffuse rhabdomyomatosis has been reported by Virchow, Uehlinger [7] and Schmincke [8] (three cases). The solitary tumors are usually located at the apical region of the heart and coexist less frequently with tuberous sclerosis than those of the multiple type. Steinbiss reported that tuberous sclerosis was present in one of four cases of solitary rhabdomyoma, but in twenty-four of twenty-eight cases of multiple rhabdomyomas. It has been noted that rhabdomyomatous foci are most frequently located in the least specialized parts of the myocardium, that is, in the subendocardial tissue. They may occur in any part of the heart and are not restricted to the location and proximity of the neuromuscular tissue.

The individual nodes may vary in size, some being recognizable only microscopically and others being larger than a walnut. While these formations may occasionally interfere with the proper functioning of the heart on account of their size and location, especially when they are located near the valvular ostia, no clinical manifestation of a disturbance of the conducting system has ever been observed.

On microscopic examination the nodes present a cavernous or spongy structure consisting of large spindle-shaped or polygonal swollen cells with large empty vacuoles and a peripheral cytoplasmic zone showing frequently more or less distinct cross-striation. The cytoplasm is often granular or foamy and possesses from fine to broad processes and bands which interconnect with adjacent cells. Delicate processes extend into the apparently empty central vacuolar spaces. The nuclei are seen to be moderately well stained and round or oval; they are located either in the peripheral cytoplasm or in the central portions of the cell and connected with the peripheral cytoplasm by cytoplasmic bands, producing a spider-web-like picture (spider cells). In larger nodes there is sometimes a tendency of the cells to be arranged in bundles. The general structure of these formations resembles in many respects that of embryonic muscle tissue at an early fetal period (thirteenth week). The content of the vacuolar spaces gives a positive reaction for glycogen with compound solution of iodine and with Best's carmine stain [9] (Abrikosoff and others).

Abrikosoff regarded the cells of more or less solid cytoplasmic appearance with peripheral fibrillar structure as the most immature type. Cells with cytoplasmic vacuoles are, according to him, degenerate forms

7. Uehlinger, E.: Virchows Arch. f. path Anat. **258**:719, 1925.

8. Schmincke, A.: Beitr. z. path. Anat. u. z. allg. Path. **70**:515, 1922.

9. Best's carmine stain is composed of carmine, 2 Gm., potassium carbonate, 1 Gm., potassium chloride, 5 Gm. and distilled water, 60 cc.

resulting from intracellular cytoplasmic disintegration, leaving in the apparently empty vacuolar spaces droplets of glycogen as products of cytoplasmic decay. The spider-like cells represent in his opinion a still more advanced type of cellular decomposition. But this conception of the developmental mechanism of the various cell forms met in the rhabdomyomatous foci has not been accepted by other investigators, who pointed out that it does not conform with the actual development of these cells, especially in regard to their glycogen content.

Abrikosoff suggested also that the cells in the rhabdomyomas are genetically related to the Purkinje cells, to which they have certain morphologic similarities, especially in regard to their glycogen content. But this interpretation has not found the support of most other investigators and appears to be unlikely for the following reasons: 1. The existence of transitional forms between the rhabdomyoma cells and the Purkinje cells has never been observed, as the nodes are always separated from these cells by fibrous tissue. 2. The rhabdomyomatous foci occur in places where normally the Purkinje cells are not found and where, therefore, the presence of cells of neuromuscular origin can be explained only by the assumption of developmental misplacement. 3. In no case has there been any clinical evidence of disturbances of the conducting system. 4. Rhabdomyomatous foci not infrequently show transitional forms between the embryonic cells of striated muscle tissue and more or less fully differentiated forms of cells. 5. The occurrence of cases of diffuse, generalized rhabdomyomatosis of the heart points to a pure muscular origin of these formations. These arguments militate also against the assumption of Bundschuh who considered the possibility that the rhabdomyomatous anlage may have occurred before a differentiation into muscular and neuromuscular elements took place.

Congenital rhabdomyomas of the heart cannot be considered as true tumors. They do not show any evidence of proliferative activity, as mitotic figures have never been seen, while, on the other hand, degenerative changes in the form of fibrosis and calcifications have been noted in older lesions (Steinbiss). The stationary nature is also emphasized by the fact that a malignant transformation has never been observed. In view of the fact that other developmental disturbances are usually present in other organs, and as in some cases the parenchymatous organs have shown evidence of pathologic enlargement, the theory that these formations are products of a surplus development of tissue due to an abnormal quality of the germ seems to be more acceptable than the explanation favored by Bundschuh and others, who have expressed the belief that the formations represent the results of mechanical developmental disturbances causing misplacement of tissue. Congenital rhabdomyomatous foci in the heart should therefore be considered as hamartomas (Albrecht) and not as choristomas (Ribbert).

REPORT OF A CASE

A case of rhabdomyomatosis of the heart is reported in support of the foregoing observations and theories and represents the first observation of this condition in a Negro.

History.—A Negro boy, aged 7 months, was admitted in December 1933 to the Graduate Hospital of the University of Pennsylvania, in the service of

Dr. Gittings, who furnished the clinical data. The child had had a normal birth and had been apparently well until 5 months of age, when he began to have "spasms" about four times a day. These attacks were tonic and were accompanied by a stare, by flexion of the arms and legs and by bending down of the chin on the chest, the patient remaining in this condition for about two seconds, then relaxing and becoming tonic again. The total duration of an individual attack was approximately five minutes. The parents noted that the child was underdeveloped mentally and that he did not sit up or take any interest in toys. He vomited frequently, but the vomiting was not of the projectile type.

Physical Examination.—Examination on admission showed a well developed Negro baby. An enlarged liver was the only abnormality noted.

Laboratory Examination.—The parents gave a negative reaction to the Kahn test. The Wassermann reaction of the blood and spinal fluid of the baby and the reaction to the tuberculin test were negative. The blood count showed 4,100,000 erythrocytes and 6,100 leukocytes; the hemoglobin was 63 per cent; there were 41 per cent neutrophils, 43 per cent lymphocytes and 5 per cent monocytes. The urine was normal. The examination of the spinal fluid showed 2 cells per cubic centimeter; the colloidal gold curve was 0000000000; the protein contents, 32.5 mg. per hundred cubic centimeters.

Course in the Hospital.—Rhinitis and otitis media developed a week after admission and were complicated three weeks later by pneumonia. This was fatal in nine days, in spite of transfusions, therapy with oxygen, injection of adult serum and general supporting measures.

Clinical Diagnosis.—The following diagnosis was made: convulsions of unknown origin, otitis media and pneumonia involving the whole right lung and the posterior portion of the upper lobe of the left lung.

Autopsy.—Macroscopic Examination: The body was that of a well nourished Negro boy about 8 months old, weighing 8,000 Gm. and measuring 75 cm. No cutaneous lesions or palpable lymph nodes were present.

The thymus weighed 18 Gm. and consisted of a pinkish-gray, granular tissue embedded in a highly edematous grayish-white turbid matrix.

The heart weighed 90 Gm. and measured 6 by 4 by 4 cm. The pericardial lining was smooth and glistening. The pericardial sac contained about 5 cc. of clear amber-colored fluid. The left ventricle was firm and contracted, and the myocardium was normal in color and appearance. The valves were delicate and intact.

The lungs showed the following conditions: The pleura of the right lung was covered with a yellow, stringy material. The upper and middle lobes were completely solidified and light pinkish gray. A yellow, purulent fluid exuded from the bronchi. The lower lobe was softer and contained numerous, small solidified areas. Numerous subpleural hemorrhagic areas were seen underneath the costal pleura. The right lung weighed 140 Gm. The pleural cavity of the left lung contained about 250 cc. of a turbid, yellow-green gelatinous material which covered the entire pleural surface, but was especially thick at the base. The posterior portion of the upper lobe of the left lung was solidified and dark reddish gray. The anterior portion was markedly distended with air and was light grayish red. The lower lobe was compressed and partially atelectatic, of fleshy consistency and dark reddish purple. The left lung weighed 70 Gm.

The liver weighed 450 Gm. and measured 16 by 11 by 6 cm. The surface was smooth and brownish red. The cut surface showed distinct yellow strands

in a reddish-brown matrix. The spleen weighed 40 Gm. and measured 8 by 5.5 by 2 cm. It had a smooth, dark reddish-gray surface and was firm. The cut surface revealed a grayish-red pulp with prominent gray follicles. The suprarenal glands weighed about 3 Gm. each and had a narrow brown medulla.

The right kidney weighed 46 Gm. and measured 6.5 by 3.5 by 2.5 cm.; the left kidney weighed 40 Gm. and measured 6 by 4 by 2 cm. The capsule stripped easily. The surface was smooth and reddish. Several small, clear cysts the size of a pinhead were visible in the superficial part of the cortex. The medulla and cortex were of normal appearance on the cut surface. The pancreas weighed 15 Gm., was firm and normal in color and texture. The stomach, intestines, bladder and testicles were normal.

The two fontanels were not closed. The dura was attached to the bony skull. The soft meninges were highly edematous and had a slightly turbid, grayish-yellow appearance. The brain was firm and gave the impression that multiple, ill defined, hard, nodular indurations were present in the cerebral cortex. The cut surface showed the presence of multiple indistinctly outlined firm areas which were of a somewhat lighter color than the surrounding gray cortical matter. At the bottom of both lateral ventricles numerous nodular, firm elevations varying from the size of a rice seed to that of a pea were found; on the cut surface these elevations appeared as small, white, ill demarcated nodules. The cerebellum appeared normal macroscopically. The brain weighed 800 Gm.

A culture from the right lung showed predominantly influenza bacilli and pneumococci (group IV).

The following diagnosis was made: confluent, bilateral bronchopneumonia, bilateral fibrinopurulent pleurisy, purulent bronchitis, fatty degeneration of the liver, septic hyperplasia of the spleen, tuberous sclerosis and multiple gliomas of the basal ganglions, and edema of the meninges.

Microscopic Examination: A microscopic examination showed the typical picture of bronchopneumonia with multiple abscesses, fibrinopurulent pleurisy, acute inflammatory hyperplasia of the peribronchial lymph nodes, acute inflammatory changes in the thymus with the presence of nests of foam cells in the interstitial tissue, active hyperemia and hyperplasia of the splenic pulp and acute seropurulent meningitis. The suprarenal glands were normal.

In addition to these acute inflammatory changes of obviously infectious origin, the brain, the pancreas, the kidneys and the heart presented evidence of the existence of multiple developmental disturbances.

Sections of the brain were stained with hematoxylin and eosin and with iron and hematoxylin. In the indurated areas of the cortex the glia cells were markedly increased and the ganglion cells considerably decreased in number. Giant cells or calcifications were not seen in the sections examined. The nodes at the bottom of the lateral ventricles consisted mainly of large, spindle-shaped, pear-shaped and polygonal cells usually irregularly arranged. In the areas where spindle cells predominated the cells were often arranged in bundles and were surrounded by a dense, delicate fibrillar network, representing cytoplasmic cellular processes. In other areas the cells were of epithelioid character and embedded in a scanty fibrillar network. These cells had a large amount of cytoplasm and moderately large round or oval, pale-stained nuclei.

A detailed description and discussion of the finer architectural changes, which were diagnosed as tuberous sclerosis with multiple spongioblastomas, a diagnosis that Dr. N. W. Winkelmann, who examined these sections, concurred in, is beyond the scope of this paper.

In the pancreas the islands were numerous and large. Here and there areas of irregular glandular formations were seen; these consisted of cells resembling morphologically the light-colored cells present in the islands. In one large area of the pancreas a dense and diffuse lymphocytic infiltration of the interstitial tissue and of the pancreatic parenchyma existed.

Several small cystic formations lined with a single layer of low cuboidal cells were found in the cortical part of each kidney. In one of these cysts a small glomerular tuft projected into the cystic lumen.

Sections were taken from three different parts of the myocardium (two from the wall of the left ventricle and one from that of the right). They were stained with hematoxylin and eosin and with iron and hematoxylin (Heidenhain), Mallory's phosphotungstic acid hematoxylin, azan carmine, Van Gieson's stain, methylene green pyronine and sudan III. Numerous foci of a loose spongy tissue were found in the myocardium. They ranged in size from small accumulations consisting of a few cells to large complexes corresponding to entire bundles of muscle cells. The outlines of these nodes were more or less well defined. But beyond the amount of connective tissue normally found around individual bundles of muscle cells there was no evidence of a definite capsular formation around the foci, which in this respect fitted well into the general structural pattern of the myocardium.

The spongy appearance of the tissue was caused by the presence of an extensive cellular vacuolation in many of the cells, which were of the type heretofore described as typical for rhabdomyomatous formations in the myocardium. A more detailed comparative examination of the structure of the various foci revealed that there existed appreciable differences in the predominant cell types found in the various nodes. In some of the nodes the cells were very large and contained a central vacuole which occupied almost the entire cell, leaving only a narrow peripheral cytoplasmic rim, which only in rare instances showed any indication of cross-striation. Other nodes were mainly composed of cells of a more solid, less vacuolated character, the vacuoles being multiple and small, and the cytoplasm of foamy appearance. While cross-striation was often present in these cells, many of them revealed only the presence of dark blue, irregularly scattered granules when stained with Mallory's phosphotungstic acid hematoxylin. The lumen of the vacuoles contained not infrequently a delicate, faintly stained, foamy material. In other nodes the majority of cells presented more or less spindle-shaped, scantily vacuolated elements showing distinct cross-striation. Double-nucleated cells were not infrequently seen. Some of the cells were very irregular in shape, possessing plump budlike cytoplasmic processes with rounded ends in which the cross-striation often ran in different directions.

Differential staining for connective tissue elements revealed a fine fibrillar network distributed between the rhabdomyoma cells. While the amount of connective tissue present in the individual nodes did not generally surpass that seen in the surrounding normal myocardial tissue, in some foci a larger amount of rather firm connective tissue was observed in the periphery of the nodes.

In a few of the foci the replacement of degenerated rhabdomyomatous tissue by connective tissue reached more advanced degrees. The main mass of the nodes consisted of fibrous tissue in which only scanty, large, highly vacuolated rhabdomyoma cells were found. In two small foci of this type calcifications were observed. They seemed to represent, according to their shape and size, solid calcium casts of degenerated rhabdomyoma cells. The peripheral zone of these calcified cells was especially dense and dark bluish purple. In a few of the cells

the calcification of the cytoplasm was not quite complete, and only numerous coarse bluish granules were present in the cytoplasm.

While the majority of the rhabdomyomatous foci were well circumscribed and could be compared to hypertrophic bundles of embryonic myocardial cells the differentiation of which had been checked for some unknown reason, as the arrangement and course of their cells and the amount and location of their connective tissue seemed to fit well into the general pattern of the surrounding myocardium, some of these foci were less definitely demarcated from the adjacent muscle tissue. In these foci strands of normal muscle cells seemed to merge with the rhabdomyomatous cells, indicating that in these nodes not an entire muscle bundle but only a part of it has been arrested in its normal maturation.

This conception received additional support from the observation of ill defined areas in the myocardium, especially often located near the endocardium and epicardium, where the muscle cells were lightly stained in their central portions, which had a granular or foamy appearance, while the peripheral zone was much denser and dark pink. Whereas cross-striation was usually present in these cells, it was less distinct than in the surrounding normal cells. In one of these areas dark pink-stained cells with a hyalinized, noncross-striated cytoplasm were diffusely scattered between the lighter-stained cells, suggesting that the developmental, inhibitory factors in these areas were not quite as strong as in the rhabdomyomatous foci.

Frozen sections stained with sudan III showed the absence of any fatty substances in the cells of the rhabdomyomatous foci, indicating that the vacuoles and the foamy appearance of the cytoplasm were not due to fat.

COMMENT AND CONCLUSIONS

The case reported is an example of a congenital, dysontogenetic malformation involving ectodermal (brain), entodermal (pancreas) and mesodermal (heart, kidney) tissues. The multiple rhabdomyomatous foci in the heart are considered as surplus formations of the myocardial tissue of hamartomatous character having no histogenetic relation to the neuromuscular elements. In addition to the arguments advanced by previous investigators [10] in support of this conception, attention may be called to the presence of disturbances in the differentiation of parts of the myocardium in ill defined areas, to differences in the degree of cellular differentiation in the various rhabdomyomatous foci and to the frequent muscle bundle-like character of some of these nodes.

The obvious morphologic similarity of some of the cells in these foci to the Purkinje cells indicates that both types of cells have preserved certain embryonic structural features which make them architecturally less specialized than ordinary myocardial cells. Rhabdomyoma cells differ, however, functionally from the Purkinje cells, which are myocardial cells specialized for conduction instead of for contraction (Krumbhaar [11]).

As the reexamination of a section from the heart in a case of tuberous sclerosis reported by Dr. Freeman [12] from the department of

10. Moenckeberg, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 2, p. 482.

11. Krumbhaar, E. B.: Personal communication to the author.

12. Freeman, W.: Arch. Neurol. & Psychiat. **8**:614, 1922.

pathology and the Hospital of the University of Pennsylvania showed the presence of multiple rhabdomyomatous foci identical with those described in the case recorded here, the total number of cases of rhabdomyomatosis of the heart so far reported is forty-five, of which only one, the case reported, occurred in a Negro.

Finally, the absence of malignant transformation of the rhabdomyomatous foci in the heart in contrast to that not infrequently occurring in congenital anlagen of this type in other organs deserves some comment. The discrepancy existing in this respect seems to suggest that the heart represents an unfavorable environmental medium for this development. A study of this phenomenon appears to be indicated, as it may throw some light on the developmental mechanism of the formation of malignant tumors in general and on that from congenital malformation of tissue in particular, as it seems possible that the constant and high metabolic activity of the cardiac muscle may play an important rôle in this matter.

General Review

THE HUMAN CORPUS LUTEUM

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INTRODUCTION

A review of the recorded studies of the human corpus luteum is fascinating, interesting, instructive and entertaining. Interest in this structure probably antedates its first recognition by description or name. Although the exact date of its discovery is not known, it probably has been observed for nearly four centuries. Knowledge of its form, relation and function, meager at first, has been greatly amplified during the past century. The present concept is not final; it is likely to be altered in the near future. At times the controversies concerning its significance have been acrimonious, and this has added considerably to the fascination of the problem. Each new discovery of fact has marked progress, while unwise inferential deductions have retarded advance. Nevertheless, sufficient factual data have accumulated to justify several important conclusions.

The exclusion of all but human material obviates many of the complexities which a consideration of species variation introduces. However, knowledge gained from experimentation and dissection of animals has often suggested the manner and the means for investigation of the human being, and therefore cannot be entirely omitted. While, on the other hand, clinical problems have stimulated new lines of research in animals, sometimes the relationship of laboratory and clinical problems is so close that the two cannot be completely divorced. But, so far as possible, the present consideration will be limited to the interpretation of the material with reference to its application to human material.

The sphere of interest includes the fields of the biologist, the anatomist, the physiologist, the pathologist and the clinician. At times, one or another of these investigators may have been dominant but, on the whole, it is the combined effort of all that has produced the present concept. The critical interpretations of each serve to stabilize the deductions made by the others, so that the continued cooperation of all is to be desired.

One of the difficulties encountered in the study of the corpus luteum is its transitory nature. It is not a fixed structure; therefore any

description must take into account its continuous and relatively rapid transformation. Furthermore, the intimate relation of the corpus luteum to the rest of the ovary, particularly to the follicles, does not always permit consideration of the corpus luteum as an entirely isolated structure.

HISTORY OF THE TERM

The present concept of the ovary and its constituents has evolved slowly over a period of approximately twenty-one centuries, but more rapidly during the present century. The trend of thought concerning this subject at any particular period during this evolution may be conjectured by enumerating some of the more conspicuous landmarks, viz., the use of the term "the female testes" by Herophilus in the third century B. C.; the discovery of follicles and corpora lutea about the middle of the sixteenth century; the application of the term "ovarium" by Fabricius in 1621, and the discovery of the human ovum by von Baer [1] in 1827. The latter event may be said to have marked the beginning of the modern history of the subject. With this began the controversy regarding the origin and structure of the corpus luteum which, even today, is not settled to the entire satisfaction of every one, although the final argument is usually conceded to Meyer [2] (1911). The concept of the corpus luteum as a gland of internal secretion announced independently at the end of the last century by von Born and Prenant [3] is of such relatively recent origin and has developed so many ramifications through an extensive literature that it would be difficult to epitomize the subject briefly. Furthermore, since the question of function is still open, consideration of this phase under "history" is hardly proper.

Returning to a more detailed consideration of the earlier history, one finds that Herophilus of Alexandria flourished at about 300 B. C. He was one of the first to perform postmortem examinations in public and has been regarded as the "father of anatomy." The ovaries of vivipara were described by him as the "female testes." "For centuries thereafter they were referred to as the testes muliebris in mammals, for they were generally conceived to be concerned in the production of the semen feminae" (Adelmann [4]).

Vesalius,[5] working with fresh postmortem material, set a standard for description of the appearance of the structure of the human body

1. von Baer, C. E.: De ovi mammalium et hominis genesi. Epistolam ad Academiam imperialem Scientiarum Petropolitanam, Lipsiae, L. Vossii, 1827.

2. Meyer, R.: Arch. f. Gynäk. **93**:354, 1911.

3. Prenant, A.: Rev. gén. d. sc. **9**:646, 1898.

4. Adelmann, H. B.: "De ovorum gallinaceorum generationis primo exordio progressuque, et pulli gallinacei creationis ordine" of Volcher Coiter, Ann. M. Hist. **5**:327, 1933.

5. Vesalius, A.: De humani corporis fabrica libri septem, Basileae, per J. Oporinum, 1555.

when viewed with the naked eye. In the second edition of his "Fabricius" (1555) he said of the "testes muliebris":

"Their external surface is uneven and, just as if many tiny glandules were bound together, it seems full of unequal tubercles." They consist of "a harder substance" (than the male testes) and are "full of follicles, not unlike the glandular bodies of the mesentery and inferior membrane of the omentum. For the female testes have within them, in addition to the vessels, certain follicles full of a slight aqueous humor, which generally springs forth as from a gushing spring to an extraordinary height on dissection if the ovary has not been previously ruptured but by force compressed, making a noise like an inflated vesicle. But as that humor in healthy women is white, resembling a dense whey of milk, so also I have discovered it to be strangely yellow and saffron-colored and somewhat denser in two very high-born girls who before their death were troubled with strangulation of the uterus and one of whom had one ovary protruding like a rather large pea full of that yellow humor of the follicles, just as in men we see the colon, where it goes past the liver, turned yellow by the gallbladder. And when the unusual color of this humor or liquid occurs, it smells very strongly, and contains something poisonous and foul in addition to itself."

Whether or not Vesalius actually saw follicles and corpora lutea, he was obviously unaware of their true nature, as shown by his illustrations, which picture the female testes quite similar in gross appearance to the male testes, even to the vas connecting them with the uterus. Note the following statement appearing in the text: "Then the vessel carrying the semen from each testis into the uterus arises from the body of the testis, just as in men; although sometimes it ascends to the testis more tortuously than in the posterior region alone."

Volcherus Coiter [6] is alleged to have been the first (1573) to note the corpus luteum. He was studying the ovary of the cow. However, he had no true conception of the function of the ovary, for he believed that the ovaries were to be compared with the seminal vesicles rather than with the testes of the male. According to Meckel, Haller and several others after him attributed the discovery of the yellow body to Coiter, but it really belongs to Fallopius,[7] who spoke of the yellow body for twelve years before Coiter, and almost in the same terms (1561). Fallopius [7] (1523-1562), the Italian anatomist who described the tubes which have since borne his name, having demonstrated that there was no true vas of the female testes, wrote of the ovary:

All anatomists assert with one voice that semen is made in the female testes, and that they are found full of semen, which I could never see although I gave no

6. Coiter, Volcherus: Externarum et internarum principalium humani corporis partium tabulae atque anatomicae exercitationes observationesque variae, novis, diversis ac artificiosissimis figuris illustratae, etc. Noribergae, in off. T. Gerlatzeni, 1573; cited by Adelmann.[4]

7. Fallopius, G.: Opera omnia . . . cui nunc demun accessit tomus secundus . . . opera et studio Johan. Petri Maphaei. Francofurti, apud. haered. A. Wecheli, 1600; cited by Barbour, A. H. F.; Edinburgh M. J. **34**:227, 1888-1889.

small labor that I might learn this. I have seen in them, indeed, some vesicles, as it were, turgid sometimes with water or a watery fluid, sometimes yellowish, sometimes limpid, but I have never seen semen except in the spermatic vessels themselves or those called "excretory." They affirm also that those spermatic vessels spring from the ovaries, and are united completely to them, and end in the so-called horns of the uterus, which is not at all the case, since I could never see these seminal tubes united to the testes, unless the uterus was pathologic.

Fabricius (1537-1619), Italian anatomist and embryologist, characterized by Singer [8] as the "effective founder of modern embryology and the author of the first illustrated work on that subject," was the first to use the term "ovarium." Adelmann [4] wrote: "The first use of the term 'ovarium' is encountered in Fabricius' description of the ovary of the hen, in 1621 . . . In the legend of his first plate he introduces the term 'ovarium' as synonymous with 'superior uterus'."

Hyrtl believed that Nicolaus Stenon [9] was the first to use the term "ovarium."

In 1668 van Horne called the follicles of the ovary, "ova." Kerkring in 1671 described what he believed to be ova in the human ovary (Adelmann [4]).

De Graaf,[10] whose first publication on this subject appeared in 1672, described in great detail the ovarian vesicles which bear his name. He wrote:

"Vesalius, Fallopius, Volcherus Coiter, Laurentius, A'Castro, Riolan, Bartholin, Wharton, Dom. de Marchettis and others have described these vesicles under various names." De Graaf, however, did not claim credit for naming the ova, for he said: "The celebrated van Horne, however, in his Prodromus, prefers calling them ova, which term, since it greatly pleases us, we shall hereafter adopt, and we shall call these vesicles 'ova' as van Horne has done by reason of the accurate resemblance they bear to the ova contained in the ovary of birds; for these, whilst they are still small, contain nothing but a certain thin liquor like albumin." De Graaf noted ova of different sizes, some being so minute as to be scarcely observable. He also distinguished between true ova and hydatids. He noted their relation to coition and considered them essential to generation. "The ova described are generated and formed in the female testes clearly in the same way that vitelli or yolks are formed in the ova of birds . . . After the ova of the testes have acquired their natural size, they are next invested with various tunics or follicles;

8. Singer, C.: A Short History of Medicine, New York, Oxford University Press, 1928.

9. Stenon, Nicolaus: Elementorum myologiae specimen, seu musculi descripto geometrica . . . Florentiae, ex typog. sub signo Stellae, 1667, p. 117; cited by Hyrtl, J.: Onomatologia anatomica. Geschichte und Kritik der anatomischen Sprache der Gegenwart, mit besonderer Berücksichtigung ihrer Barbarismen, Widersinnigkeiten, Tropen und grammatikalischen Fehler, Vienna, W. Braumüller, 1880.

10. de Graaf, R.: On the Female Testes, or Ovaria, translated from the original edition of 1688 by R. Knox. Bound with Clay, C.: British Record of Obstetric Medicine and Surgery, Manchester, W. Irwin, 1848, vol. 1.

between which, immediately after coition, a certain glandular matter grows much up (appears and increases much, and suddenly swells up); of which matter the substance of the globes just described is composed. . . . The common use then of the testes of the female is to generate ova, and to nourish them and bring them to maturity; so that they perform the same functions in women as the ova of birds. Hence, they ought rather to be called ovaria than testes since they resemble neither in form nor substance the male testes properly, so called; for which reason they were considered by many as useless bodies, an opinion evidently incorrect, seeing that they are, in the highest degree, essential to generation."

Although corpora lutea were not so named by him, de Graaf noted that the globules which were present only after coitus corresponded to the number of fetuses. A few days after coitus these globules contained a limpid fluid which, in the later months of pregnancy, became solid, and after parturition, disappeared. He was chiefly concerned with establishing that mammals ovulate.

Malpighi [11] in 1686 named the structure found in virgins the "corpus luteum," but he believed that it was preformed in the ovary, and that it gave rise to the follicle and the ovum. He compared it to a gland and attributed to it the function of nourishing the ovum. In spite of Malpighi's imperfect conception of the structure of this body and the insufficiency of the term as indicating all its stages, the designation "corpus luteum" is so ingrained in the literature that it has never been displaced by any more accurately descriptive name. For instance, Robin [12] supplied a new name for the corpus luteum, "ovariule," by which he wished to imply that it was a scar of the ovary, this name being derived from two Greek words which gave that meaning. This new term, although noted by a few contemporary authors, was always used parenthetically. The work of Malpighi is so remarkable that it is quoted at length:

After a time, a solid yellow body emerges and increases to such a comparatively huge size that it projects like a sort of papilla on the whole ovary; its size, after complete growth, equals that of a cherry. Its external surface is rough and dotted with several small elevations of unequal size; it is surrounded by fleshy fibers which, as is observed in glands, insinuate themselves inward. It is also traversed by blood vessels and nerves. The membrane is involved particularly in the papillary appendage, which the tunica communis ovarii further invests. The formation, when incised, is found to be composed of small, almost lobular parts, as we have observed in other viscera: they are, however, full of angulations and proceed in various directions; they appear to be attached to the blood vessels and protracted like an umbilicus. The line separating the lobes is indistinct, and is formed by varicose offshoots of yellow color, on which subspherical bodies and appendages of gold color are seen, as if tiny particles of fat were joined together and continuous. The interior configuration of the yellow body when dissected was not

11. Malpighi, M.: Opera omnia, seu thesaurus locupletissimus botanico-medico-anatomicus, Lugduni Batavorum, P. Vander Aa, 1687.

12. Robin, C.: Compt. rend. Soc. de biol. **3**:139, 1857.

found to be exactly the same from one case to the next, but occurred in varied forms. Sometimes, for example, to speak more explicitly, this yellow body is found packed tightly together and enveloped by varicose productions of a yellow or sometimes grayish color, and seems to me scarcely to exceed the size of a grain or that of a vetch seed. More frequently around the vesicles swollen with viscous fluid, or the albumin of the ovum, and hitherto very small, was drawn a cover confining the yellow substance and extending somewhat like a tendril. Not infrequently this yellow body scarcely reaches the size of a chick-pea, assumes the shape of a pear, and inside at the center toward the gradually narrowed neck, is swollen with serous fluid. It often acquires the size of a cereal grain, pushing the papilla outward from the ovary; it contains in the center the vesicle swollen with viscous fluid, like drops of cerasin; the vesicle is sometimes round, and not rarely is endowed with several rod-shaped appendages, but more frequently has only one. In this body, yellow throughout, there is sometimes contained almost no viscous fluid, but very frequently from the tunica covering the papilla on the inside, where the pit on the outside is stimulated to the utmost, and the foramen is finally hollowed out, a certain ashy membranous, and perhaps fistulous, body is produced which, elongated perpendicularly toward the center, is as though divided into the branches of vessels, which project into the whole structure of the yellow body, and these lobules are appended in various directions. In certain adult yellow bodies toward the center, and whenever the ovule with an appendage the size of a millet-seed is observed more deeply, it is stretched by this wrinkled, grayish body. Often a sort of meatus or duct is opened and is prolonged inward as far as the center of the papilla, in which a clear humor stands, which heat hardens into albumin, and not rarely the ovule, or both given appendixes are preserved, not very greatly dissimilar in form from those which are observed in oak-galls. And finally, these wornout yellow bodies are thrown off. Through a winding duct which leads out to the middle of the papilla a stylus can be introduced; the cavity enclosed is the size of a pea, and is lined by one enveloping membrane which is continuous with the duct. And thus these bodies, found in various forms, are seen to demonstrate probably that this yellow body acts not only as a protection for the ovule, whose ejection may be stimulated by nature, but perhaps contributes to generation itself, and hence might be considered glandular rather than muscular; for its structure is not fibrous, or fleshy, but rather similar to the accessory kidneys; whence it probably may be suspected that this yellow glandular substance is made by filtration, and the material is changed into the ovule, or foregoing scar filled with ichor. We observe a production analogous to this in the seeds of plants, in which the umbilical vessel first emerges, the end of which gradually becomes loosened, and swells out with the infiltrated viscous fluid, the young sprout finally taking form. For these reasons, also, it may be doubted, that the vesicles, many of which for a certain time flourish in the ovaries, endowed by chance with unequal size and full of viscous fluid which can be hardened, are true ova which subsequently become fecund, but perhaps are material from which the glandular yellow body first develops. Indeed, it is seen not only after coitus and effusion of male semen that this yellow and glandular substance appears and these signs of the fecundated enclosed ovum are presented; for very frequently in new-born calves, one or another conspicuous vesicle is discovered, from which this yellow grainlike substance is born in addition. Also in cattle at a certain stage of pregnancy and with the contained fetus at various stages, I observed these yellow bodies, sometimes equal in size to a chick-pea, sometimes to a cereal grain, and very numerous, in the ovaries; but this did not lead to a suspicion of superfetation. This same thing I have observed in women at about the seventh month of pregnancy, and indeed in a single ovary in various animals there were present several of these yellow bodies of unequal size

in which multiple feti did not follow. I might add that in several of these ovaries, studied with especial care, the vessels were swollen, being filled with hardened yellow fluid. It may also be doubted that only one vesicle of albumin is consumed for the generation of one yellow body, but perhaps several take part; for where a perfect yellow body swells out it occupies not nearly the whole concavity of the ovary, but frequently few vesicles of albumin remain, and at other times these are very numerous. Therefore, considering these not improbably associated, this yellow and glandular substance does not immediately follow the effusion of semen into the ovum contained in the ovary; but precedes it by a long time and assists in delivering the ova; in like manner vesicles filled with viscous fluid are not hard ova, but matter by which the gland is stimulated, or whose ovum is detached, protected and stimulated at the appointed time.

Von Baer [1] stated that "many anatomists in the seventeenth century sought in vain for the ova of mammals in the tubes, but nevertheless under the leadership of the acute Philip Jacob Hartmann of Königsberg they denied all faith in the observations of Regnier de Graaf."

Concerning the presence of the corpus luteum only in virgins, Malpighi's observations were confirmed by Vallisnieri, Santorini and Buffon (Mulon [13]).

During the eighteenth and early nineteenth centuries, many lively and spirited debates were recorded on the question whether impregnation could be inferred with certainty if the presence of a corpus luteum was established.

Haller [14] in 1765 was the first to describe accurately the transformation of the graafian follicle into the corpus luteum; however, he believed that the latter never existed except as a consequence of impregnation.

It remained for von Baer [1] in 1827 to clarify the relation between the ruptured follicle and the corpus luteum. He said: "I found the origin of the ovum of mammals and man in the ovary, which had been sought in vain for so many centuries and after much labor." His material consisted chiefly of dogs, but he made observations on many other animals with two brief references to the human being. In one case he saw the "internal stratum of the theca of the vesicle still closed"; in the other, "the corpus luteum was open." "The cavity . . . was greater than the corpus luteum of another animal." He found the origin of the corpus luteum in the theca folliculi.

Up to the time of the epoch-making work of von Baer, the problem remained relatively simple, as the various investigators were chiefly concerned with the macroscopic presence of the corpus luteum and, to a certain extent, with its relation to coition and pregnancy. After von Baer's detailed studies, new problems arose, which included the gross

13. Mulon, C.: Ann. de gynéc. et d'obst. **72**:415, 1917.

14. Haller, A.: First Lines of Physiology, ed. 1, translated from the third Latin ed., Troy, N. Y., O. Penniman & Co., 1803.

and the histologic structure and form, the relation to the rupture of the follicle, the relation to menstruation and pregnancy, the function, the chemical content and elaboration of hormones, and the abnormalities. Many times these problems were studied concurrently, so that it is difficult to epitomize the work on them historically. It seems simpler, therefore, to trace the evolution of each phase of the subject separately.

ORIGIN OF THE CORPUS LUTEUM

Interpretation of the origin of the corpus luteum was concerned first with the cause of its existence, and this interest soon developed into a question of its relation to impregnation. Later, "origin" referred to its tissue derivation.

In the matter of the origin of corpora lutea, all the earlier writers were chiefly interested in whether these structures ever occurred without impregnation. For a time the belief in this causal relationship was very strong. Haller[14] (1765), who was the first to describe accurately the origin of the corpus luteum from the transformation of a recently ruptured graafian follicle, denied its existence in virgins. Meckel[15] (1825), Velpeau[16] (1831), Montgomery[17] (1837) and many others wrote of corpora lutea as occurring only after coitus. According to Corner,[18] "Abernethy and Sir Astley Cooper swore away the innocence of a dead woman in a court of law because one of her ovaries was found at post-mortem to contain a corpus luteum."

Concurrent with those who believed that corpora lutea occurred only as a result of coitus were many other writers cited by various authors, e. g., Malpighi, Valisnieri, Santorini, Brugnone, Bertrandi, Buffon and others, who noted corpora lutea in virgins. However, these structures unassociated with pregnancy were looked on as abnormal or pathologic formations, hence the designation "false corpora lutea."

True and False Corpora Lutea.—The "false" corpus luteum is referred to in the literature also as the "corpus luteum menstruationis, or spurium"; and likewise, the "true" corpus luteum is referred to also as the "corpus luteum of pregnancy, graviditatis, or vera." (The terms designating true corpora lutea may be used interchangeably; the

15. Meckel, J. F.: Manuel d'anatomie générale, descriptive et pathologique, translated from the German by A. J. L. Jourdan and G. G. Breschet, Paris, J.-B. Ballière, 1825, vol. 3, p. 735.

16. Velpeau, A. A. L. M.: An Elementary Treatise on Midwifery; or Principles of Tokology and Embryology, translated from the French by C. D. Meigs, Philadelphia, J. Grigg, 1831; cited by Dalton, J. C.: Tr. A. M. A. **4**:549, 1851.

17. Montgomery, W. F.: An Exposition of the Signs and Symptoms of Pregnancy, the Period of Human Gestation and the Signs of Delivery, London, Sherwood, Gilbert & Piper, 1837; cited by Dalton, J. C.: Tr. A. M. A. **4**:549, 1851.

18. Corner, G. W.: The Corpus Luteum of Pregnancy, as It is in Swine, Contrib. Embryol. no. 222, Carnegie Institution of Washington, 1915.

designations are synonyms. The same may be said of the terms referring to false corpora lutea.) Some earlier observers noted correctly the presence of corpora lutea independent of coition or impregnation, but either ignored the facts or tried to explain the occurrence on other grounds. Gradually the true state of affairs dawned on them, and the foundation was laid for a new controversy, viz., whether there was any difference between the true and the false corpora lutea. The culmination of this debate was reached in the early part of the nineteenth century.

From the earliest to the present observations the association of the corpus luteum with pregnancy has never been seriously questioned. The main contention has been regarding the relation between the false corpus luteum and menstruation. Many of the differences of opinion are to be explained by errors in inferences by analogy in which it has been assumed that menstruation of the human being is like rut in animals. Furthermore, prior to the introduction of asepsis in surgical operations, observations were limited to material obtained at autopsy. Even with the advent of observations on surgical material very little of practical value was contributed toward the solution of this problem until the classic experiments of Fraenkel [19] in 1903.

The establishment of the present concept of the corpus luteum of menstruation can be attributed largely to the painstaking efforts of Meyer [2] (1911), who demonstrated the stages of development from the rupture of the follicle to completion at the time of menstruation as well as the involution.

Interest in the distinction between the true and the false corpora lutea concerns the following phases: (1) the possibility of the existence of the structure without impregnation or coitus; (2) the relation to the rupture of the follicle, including the manner of healing without scar; (3) the comparison of, first, the gross, and, later, the histologic structure; (4) the function. Each of these phases will be considered in detail in other sections.

Beginning with von Baer,[1] a new line of investigation was started with reference to the origin of the corpus luteum. He maintained not only that it arose in consequence of a ruptured follicle, but that it was derived from the theca folliculi. Later Bischoff [20] demonstrated its origin from the cells of the membrana granulosa. Each of the proponents has had many ardent supporters, but today the great majority of observers favor the origin from the cells of the membrana granulosa, and there are only a few supporters of the thecal origin. The latter are influenced by the findings in various species of animals.

19. Fraenkel, L.: Arch. f. Gynäk. **68**:438, 1903; **91**:705, 1910.

20. Bischoff, T. L. W.: Entwickelungsgeschichte des Kaninchen-Eies, Braunschweig, F. Vieweg & Sohn, 1842.

Some exceptions to these theories are to be noted, which have never been widely accepted. Thus, Malpighi [11] believed that the corpus luteum was preformed, and that in its development it gave rise to the ovum and the follicle. Home [21] also believed that ova were formed in the corpora lutea. Négrier,[22] while considering that the corpora lutea were always formed as a result of the rupture of the follicles, supposed that the yellow matter was deposited between the coats of the follicle prior to its rupture, and that the yellow matter, as well as the effused blood, was afterward rapidly absorbed. This view was partially supported by Paterson [23] (1840), who considered the true corpus luteum as arising from a deposit between the layers of the graafian vesicles. However, he described the false corpus luteum as arising from a more or less complete filling of a graafian vesicle with blood, or by an undefined effusion of blood into the substance of the ovary. His conception of a metamorphosis of the blood poured into the cavity of the ruptured follicle without any particular participation of the follicular membrane was confirmed by Henle [24] in 1841.

Waldeyer [25] in 1870, assuming a middle ground, attributed an equal share in the formation of the corpus luteum to the membrana granulosa and to the theca interna, first one predominating, then the other.

Returning to the two main theories of origin, i. e., those relating to the membrana granulosa and the theca, one finds the alleged adherents of each prior to 1899 tabulated by Clark [26] as follows:

Supporters of von Baer's Theory (1827): The Corpus Luteum Arises from the Theca Interna		Supporters of von Bischoff's Theory (1842): The Corpus Luteum Arises from the Follicular Epithelium
Valentine, 1835	Benckiser, 1884	R. Wagner
Leuckart	Paladino, 1887	Meckel von Hemsbach, 1852
Zwicky, 1844	Nagel, 1888	Pfluger, 1863
His, 1865	Bonnet, 1891	Schroen, 1863
von Rokitansky, 1861	Crety, 1893	Luschka, 1864
Spiegelberg, 1865	Schottlaender, 1893	Funke, 1866
von Kölliker, 1867	Hoelzl, 1893	Waldeyer, 1870
Slavjansky, 1870	Minot, 1894	Call and Exner, 1874
Beulin, 1870	Clark, 1897	Beigel, 1878
Gegenbaur, 1883		G. R. Wagener, 1879
		Schulin, 1880
		Sobotta, 1896-1897

Following this review, Clark, working in Spalteholz' laboratory, employed a special method of tissue digestion devised for the isolation

21. Home, E.: Phil. Tr., London **107**:252, 1817; **109**:59, 1819.

22. Négrier, C.: Recherches anatomiques et physiologiques sur les ovaires dans l'espèce humaine, considérés spécialement sous le rapport de leur influence dans la menstruation, Paris, Bechet jeune & Labé, 1840.

23. Paterson, R.: Edinburgh M. & S. J. **53**:49, 1840.

24. Henle, F. G. J.: Allgemeine Anatomie: Lehre von den Mischungs- und Formbestandtheilen des menschlichen Körpers, Leipzig, Leopold Voss, 1841.

25. Waldeyer, H. W. G.: Eierstock und Ei: Ein Beitrag zur Anatomie und Entwicklungsgeschichte der Sexualorgane, Leipzig, Wilhelm Engelmann, 1870.

26. Clark, J. G.: Johns Hopkins Hosp. Rep. **7**:181, 1898.

of the connective tissue. His material was taken from the sow and later correlated with human corpora, the results of his investigations in the animal being used as a means to identify the various stages of development of the human corpus. His conclusions led him to adhere to the thecal origin. His position can be explained by his use of a method to isolate the connective tissue and his choice of the sow for his analogies, for the theca, as shown later by Corner,[27] assumes an importance in the sow which is not characteristic of it in the human being.

Sobotta's intensive study of the corpus luteum of the mouse [28] and other mammals profoundly influenced subsequent considerations of the human corpus luteum.

Fraenkel,[19] although chiefly concerned with the function of the corpus luteum, favored the origin of the lutein cells from the membrana granulosa.

Although various stages of the corpus luteum were studied by His,[29] Benckiser [30] and others, it remained for Meyer [2] to describe in detail the macroscopic and the microscopic appearance of the stages from the ruptured follicle to complete involution, and to relate them to the cyclic changes in the uterus. His thorough investigation established the origin of the corpus luteum from the cells of the membrana granulosa, and since then his position, so far as human material is concerned, has seldom been questioned. Therefore, the opinion generally held today is that the human lutein cells are derived from the membrana granulosa.

Divergence of opinion regarding the origin of the human lutein cell can be explained now by differing definitions of what constitutes a lutein cell. There is general agreement that activity (mitosis and the storage of fat) of certain cells of the theca may be observed in the mature follicle, a process which continues into an early stage of the corpus. Hence the term "thecal lutein cell." However, early in the development the lutein cell derived from the membrana granulosa becomes dominant and is the cell usually described as the lutein cell.

MACROSCOPIC STRUCTURE

Microscopic technic for studying tissues was developed so long after the corpus luteum was discovered that it is only natural that the earlier observers described only its gross appearance. Schwann's theory of the cell structure of animals appeared in 1838-1839 and Virchow's "Cellular

27. Corner, G. W., in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1928, vol. 2.

28. Sobotta, J.: Arch. f. mikr. Anat. **47**:261, 1896; Anat. Hefte **8**:469, 1897.

29. His, W.: Arch. f. mikr. Anat. **1**:151, 1865.

30. Benckiser, A.: Arch. f. Gynäk. **23**:350, 1884.

Pathology" in 1856; therefore histologic descriptions could not be expected before these dates. Furthermore, most of the earlier material was obtained at autopsies.

Size.—Variation in the size of the corpus luteum has been noted from the first. Thus Malpighi [11] wrote: "Its size after complete growth equals that of a cherry. . . . Not infrequently this yellow body scarcely reaches the size of a chick-pea. . . . It often acquires the size of a cereal grain." Meckel [18] wrote of the corpus luteum that it was "nearly the size of a cherry." Coste [31] described it as of "the size of an ordinary nut." Robin [12] wrote: "It varies in size from that of a hazelnut to even that of a large pinhead." The objects to which the corpus luteum has been compared in size most frequently are the pea and the cherry.

Dalton,[32] in reporting on a series of twenty-six observations, explained the variation in size by the ages of the patients and by the condition of the vascular system generally. He indicated the lutein body to be "about as large as the end of the little finger" and gave accurate measurements: "three-fourths by three-eighths of an inch" and "five-eighths of an inch in its long diameter." One ovary described by him contained four old corpora lutea "the largest of which was three-sixteenths of an inch in diameter."

Seitz [33] reported measurements in fourteen cases which averaged 1.5 by 1.1 by 0.6 cm.

In twenty-eight specimens measured at the operating table, I found an average size of 1.4 by 1.2 by 0.83 cm. Most of these represented the latter half of the intermenstrual period. Eleven of them had an average fluid content of 1.4 cc. The average weight of seventeen was 2.1 Gm. Allen and his collaborators [34] found the average weight of eleven corpora taken shortly after the rupture of the follicles to be 1.25 Gm.

It is generally agreed that there is variation in size according to the development; the corpora at the stage of involution are the smallest, while the largest are found during the stage of maturity and during pregnancy. However, besides this, there is considerable individual variation which has not been satisfactorily explained.

Shape.—No particular importance has been attached to the shape of the corpus luteum. Most frequently it is found to be ovoid or

31. Coste, J. J. M. C. V.: Histoire générale et particulière du développement des corps organisés, publiée sous les auspices de M. Villemain, Paris, V. Masson, 1847-1859.

32. Dalton, J. C., Jr.: Tr. A. M. A. **4**:549, 1851.

33. Seitz, L.: Arch. f. Gynäk. **77**:203, 1906.

34. Allen, E.; Pratt, J. P.; Newell, Q. U., and Bland, L. J.: Am. J. Physiol. **92**:127, 1930.

rounded. Thus Malpighi mentioned "the shape of a pear." Dalton described it as "ovoid" and "globular rounded." Although generally it assumes a rounded form, whenever pressure is exerted by surrounding structures the corpus luteum may share in the results of this pressure and be flattened. On cross-sections many observers have been impressed by the folded appearance of the wall.

Consistency.—The varying stages encountered in the evolution of the corpus luteum necessarily lead to variation in the description of its consistency. Thus, the earlier stages are often spoken of as soft and doughy. Many have been impressed by the cystic character of the structure. Concerning the later stages, particularly the period of regression, there is more uniformity of opinion in that the structure is almost always said to be firm. The friable nature of the substance on section has been frequently observed. The fact that it has been so frequently shelled out of a definite capsule indicates the usual consistency.

Color.—As the corpus luteum was so named on account of its color, one might assume that it is always yellow. However, with the extension of knowledge of the duration of the corpus luteum, it was found that in the earlier stages there is no yellow color by macroscopic observation. The shade of yellow depends on the amount of lipochrome, which increases as the structure matures. This will be considered further in the description of the individual cell and of its chemical content. The amount of blood accounts for shades varying from red to blue or even black.

Cross-Section.—After rupture of the follicle, the walls of the cavity collapse, and the membrana granulosa already in proliferation appears as a layer about 0.25 to 0.5 mm. in thickness. After the rupture point is blocked, follicular fluid accumulates, in spite of which the lutein wall is not usually again expanded but becomes folded as a sign of the greater growth of the lutein layer in comparison with the surroundings. At the time of beginning vascularization the folding is still insignificant, but gradually the wavelike mounds become higher and more compressed. The lutein layer then measures from 1 to 1.5 mm., but on account of the folding it appears grossly to be from 2 to 3 mm. in thickness. The coagulum in the cavity is at first easily removed, but later, through organization, it becomes firmly fixed. Only at times does the content of the cavity remain fluid, and a cavity persists even during involution up to the corpus albicans. The fresh lutein layer rises slightly above the cut surface, even more in the corpus luteum of pregnancy. As a rule, at the end of menstruation the cavity disappears, and the space becomes flat with parallel walls. The tissue becomes more hyaline, tough, white and opaque. In older women numerous corpora albicantia remain.

MICROSCOPIC STRUCTURE

The observations of all the earlier writers were necessarily limited to the gross structure. Even after histologic preparations were available, it was a long time before suitable material was obtainable, for before the days of aseptic surgical operations, only postmortem specimens were studied. Then came a period of brilliant studies of animal ovaries occasionally supplemented by meager reports on human material. The research in animals was stimulating but often led to unreliable deductions concerning human tissue. Furthermore, illustrations of the early stages of the corpus luteum, when the yellow material was scant, were conspicuously absent. Therefore, the importance of Meyer's [2] contribution in 1911 is readily appreciated since he was the first to publish a comprehensive study of the corpus luteum in all the stages of its evolution from rupture of the follicle to regression. No doubt he was influenced by the intensive investigations of Sobotta [28] on the ovaries of mammals, beginning with the mouse (1896), as well as by the new interest in the function of the corpus luteum, originating with Prenant [3] and with von Born's unpublished theory transmitted by his students. Curiosity concerning the function of the corpus luteum perceptibly influenced the study of its structure during the period from 1898 to 1914. (Since then, interest in the function has been dominant, and morphologic investigations have been forced more and more into the background.) The alluring subject was approached from various angles by different observers.

Thus, Hitschmann and Adler [35] in 1908 revived and further elucidated the problem of the cyclic changes in the endometrium (the study of which was begun by Kundrat and Engelmann in 1873), thereby laying a good foundation for the correlation of cyclic endometrial changes with the stages of the corpus luteum undertaken by Meyer and Ruge.[36] This correlation was furthered by the works of Schröder [37] and Miller.[38] The conceptions of these authors were corroborated and summarized by Frank [39] in 1914.

These fundamental contributions so satisfactorily established the stages of development of the corpus luteum that only slight amendments have been necessary since that time. Briefly, the stages are: (1) proliferation, (2) vascularization, (3) maturity, (4) regression (5) corpus luteum of pregnancy. Although the stages are relatively distinct, a

35. Hitschmann, F., and Adler, L.: Monatschr. f. Geburtsh. u. Gynäk. **27**:1, 1908.

36. Meyer, R., and Ruge, C.: Zentralbl. f. Gynäk. **37**:50, 1913.

37. Schröder, R.: Arch. f. Gynäk. **101**:1, 1913.

38. Miller, J. W.: Arch. f. Gynäk. **101**:568, 1914.

39. Frank, R. T.: Surg., Gynec. & Obst. **19**:618, 1914.

sharp separation is often not possible. The appearance at the individual stages may be characterized as follows:

Maturity of Follicle and Ovulation.—As a preface to the consideration of the individual stages of the corpus luteum, a brief consideration will be given to the mature follicle, which is the precursor of the first stage. First of all, it is necessary to determine what constitutes a normal mature follicle. While the size of the follicle, the activity of its growth and the number of its cells indicate approaching maturity, the determinative criterion is the maturation of the ovum. The material for the study of the mature human ova was that taken at autopsies up to the time of Nagel [40] (1888). His excellent description of an ovum taken from operative material is still used in many texts as an illustration of a mature ovum. In the ovum which he described the nucleus was centrally located and maturation division had not begun. It is now known that prior to miosis the nucleus is peripherally located.

Apparently there are only four observations recorded in the literature concerning the division of human ovarian ova at maturation.[41] The most convincing of these is the last,[41d] in which an oocyte is described in the process of miotic division, having been recovered from a 10 mm. follicle on the fourteenth day after the onset of menstruation. The chromosomes of the second maturation spindle were peripherally located. At least twenty-two were counted. Twelve chromosomes were counted in the first polar body. The follicular cells of the cumulus oophorus showed active growth, indicated by mitosis. Considering the number of investigations undertaken for this purpose, one finds it remarkable that other successful attempts have not been reported. However, this single instance serves as verification of the fairly numerous descriptions of the appearance of a follicle approaching maturity. (Mention should be made of Stieve's [42] report of an ovum in first maturation division in a 14 mm. slightly atretic follicle found on the twenty-third day of a regular twenty-eight day menstrual cycle. Atretic follicles do not lead to the formation of corpora lutea; therefore this case is not pertinent.)

The size and number of follicles in the ovary are subject to considerable variation. There may be from one to thirty-five or more large follicles present at one time. Arnold [43] counted eighty-eight in one ovary. The average size of normal mature follicles may be placed

40. Nagel, W.: Arch. f. mikr. Anat. **31**:342, 1887-1888.

41. (*a*) Thomson, A.: J. Anat. **53**:172, 1918-1919. (*b*) Dixon, A. F.: Irish J. M. Sc. **6**:149, 1927. (*c*) Hoadley, L., and Simons, D.: Am. J. Anat. **41**: 497, 1928. (*d*) Allen, E.; Pratt, J. P.; Newell, Q. U., and Bland, L. J.: Am. J. Anat. **46**:1, 1930.

42. Stieve, H.: Jahrb. f. Morphol. u. mikr. Anat. (Abt. 2) **6**:351, 1926.

43. Arnold, L.: Anat. Rec. **6**:413, 1912.

at from 10 to 15 mm., which corresponds very well with the average of from 15 to 18 mm. for the corpus lutea. Much wider limits than those of the average figures are possible. Many of the large follicles visible on the surface of the ovary appear normal grossly, but on subsequent histologic examination are found to be atretic.

Activity of growth is attested by mitoses, increase in the number and size of cells and lack of any degenerative signs in the cell. Multiplication of the cells of the membrana granulosa, indicated by numerous mitoses, continues until the time of ovulation, then suddenly ceases and is observed only occasionally after that time. Mature follicles are lined by three or more layers of club-shaped, round or polygonal granulosa cells which are closely packed together. These cells are heaped up in one place, forming the cumulus which surrounds the ovum. Prior to ovulation the cumulus is loosened by normal processes of growth so that at the time of rupture the ovum, surrounded by granulosa cells, floats out through the opening. Most observers do not agree with Shaffer, Strassmann and Shaw that the cumulus rotates to lie near the point of future rupture. Further evidence of active growth is seen in the secretion of the granulosa cells, which forms the clear follicular fluid. The finding of cloudy or gelatinous fluid is evidence of atresia.

According to Shaw,[44] there are two well marked changes in the theca interna, during the ripening of the follicle: (1) the production of new capillaries among the cells of the theca interna which is not shared by the membrana granulosa; (2) the hypertrophy of the cells of the theca interna so that at the end of the ripening of the follicle and also immediately after ovulation they surpass the granulosa cells in size. The theca is found to form a continuous layer with the exception of irregularities and gaps due to pressure produced by adjacent structures. The theca interna is distinct from the membrana granulosa and usually marked by a limiting membrane, but its separation from the theca externa is not always sharp. In the thecal cells a difference from other connective tissue cells is noted in the lack of fibrils; however, there are fine fibrils between the cells. Storage of fat is noted in the thecal cells and to a lesser degree in the cells of the membrana granulosa. Mitoses are present in the thecal cells.

Ovulation.—The actual rupture of the follicle has never been observed in the human being, so that reports concerning this process are either theoretical or inferential, and observations are limited to specimens seen before and after rupture. The cause of ovulation is still a matter of theory. However, the important thing here is that ovulation marks the beginning of the corpus luteum. The time of ovulation in the cycle will be discussed later.

44. Shaw, W.: J. Obst. & Gynæc. Brit. Emp. **34**:300, 1927.

Atretic Follicles.—Before continuing with the description of the development of the corpus luteum, a brief account of the follicles not reaching maturity will be given, since they sometimes contain structures which resemble lutein cells although a true corpus luteum is not formed. As soon as the impulse to follicular growth ceases, the process of atresia begins. As atresia may begin at any stage in the development of the follicle, it is not surprising that the pictures presented by atretic follicles vary rather widely. According to Seitz,[38] who reviewed the literature extensively and contributed investigations of his own, this process in the follicle of an ovary is essentially the same whether it occurs before puberty, during pregnancy or during the menstrual cycle.

In some cases the ovum shows signs of degeneration before the other elements of the follicle are altered, but often the ovum remains unchanged until its investments have degenerated. Early atresia may be difficult to diagnose, but further advanced degeneration of the ovum is evident [41d] in an eccentric position of the nucleus, crinkling of the nuclear membrane, disappearance of the nucleolus or even of the whole nucleus, and vacuolation, fragmentation or segregation of constituents of the cytoplasm. Another form of advanced atresia is evident in an invasion of cells under the zona pellucida. The follicular fluid surrounding the ovum is cloudy or gelatinous.

For the most part the diagnosis of atresia has been made from a study of the membrana granulosa and theca. The latter is often edematous. Normally, the whole follicle shrinks, the membrana granulosa degenerates and subsequently disappears, and the theca cells at first hypertrophy and then are transformed into a fibrous mass—the corpus candicans. The granulosa cells are not present in as many layers as are found in a normal follicle; however, as pointed out by Meyer [45] in 1913, there may be islands of cells which are difficult to distinguish from the true lutein cells of the corpus luteum. Both the granulosa and the thecal cells may contain lipoids which apparently are similar to the lipoids of the lutein cells found in the corpus luteum.

The designation of an atretic follicle as an accessory follicular or lutein gland is not amiss, for histologically the cells sometimes appear active; furthermore, active estrogenic substance has been extracted from atretic follicles.

Proliferation or Hyperemic Stage.—The morphologic changes begun before ovulation are continued. There is increased vascularity of the theca externa and interna, indicated by widening of the capillaries, the process being more extensive in the externa. Storage of fat occurs in the cells of the theca interna and membrana granulosa, more noticeable in the former. The granulosa layer becomes stratified and is usually

45. Meyer, R.: Arch. f. Gynäk. **100**:1, 1913.

three or more cells in thickness. The follicular cavity is empty; when the opening is plugged, it becomes redistended by the secretion of granulosa cells. Mitoses, which were frequent in the granulosa cells of the mature follicle, become less evident or are absent. In the theca interna mitoses may be still present.

After expulsion of the ovum the stigma is almost immediately closed by the plasma plug. At the same time, owing to the sudden release of pressure within the follicle and the sudden diminution in its dimensions, the follicle layers evert. The former granulosa cells become matted together with the plasma plug, and on account of the hypertrophy of the cells the granulosa layer soon becomes continuous across the stigma. This is usually followed shortly by growth of the connective tissue cells. The rapidity with which the stigma is covered varies considerably, and in case of hemorrhage into the follicle it may remain patent for some time. At any rate, the site of the stigma is discernible for some time even after it is covered. Hemorrhage into the follicle is not the rule, because capillaries do not ordinarily extend into the membrana granulosa at or before rupture. Ordinarily the closure of the stigma occurs rapidly, but in some instances the membrana granulosa and theca are poorly developed at this point, allowing the stigma to be closed only by a fibrin plug for some time.

Hemorrhages from the capillaries in the thecal layer are occasionally sufficient to give rise to symptoms of local irritation, and thereby become pathologic. Not a few women are aware of the occurrence of ovulation by a sensation of slight discomfort in the region of one or the other ovary, and if examination is made at these times the corresponding ovary can be felt to be slightly enlarged and irregular and the examination reveals increased tenderness. Some patients with such discomfort have kept records of this process for several successive months.

Vascularization Stage.—In the second or vascularization stage, the theca externa is still quite vascular. The theca interna is less prominent, while the former membrana granulosa increases conspicuously by hypertrophy of the cells. The central coagulum is thin or gelatinous and may or may not contain blood. Organization of the former membrana granulosa occurs by blood, endothelial cells and fibroblasts from the capillaries and from the theca interna penetrating through the spaces between the cells to the central coagulum, the capillaries forming a lining network. Fibroblasts have been found in the central coagulum within forty-eight hours after ovulation. The storage of the lipoid and lutein in the granulosa cells increases their size. The nuclei are spherical and stain less deeply. The folding of the former membrana granulosa and theca interna begun in the first stage is now increased. The folded appearance of the corpus luteum is partly due to release of pressure but

for the most part is the result of hypertrophy of the granulosa cells. The thecal cells are already shrunken and no longer appear coherent, and are chiefly found in the connective tissue septums of the folds.

Mature Stage.—The third or mature stage is the one most frequently described as typical for the corpus luteum. The theca interna is barely distinguishable from the theca externa. The derivatives from the membrana granulosa predominate, while the increase of the capillary network spreading over them smoothly lines the inner contour of that layer. Connective tissue organization occurs in the central coagulum, or this becomes cystic, or it is a hematoma. The individual cells of the theca are insignificant and decrease in number. Most of the thecal cells are grouped in the septums of the larger folds of the granulosa derivative. The uniform lutein cells are larger and irregularly cuboidal, with light spherical nuclei. Along with their increased fat content blood pigment may be seen. Firm capillaries, accompanied by a few spindle cells, penetrate between the lutein cells, and the individual lutein cells are surrounded by fibrils. The vessels passing through the theca are well developed, indicating the inactivity of this layer.

Involution.—The fourth stage, that of involution or regression, is characterized by a predominance of connective tissue, the lutein cells becoming smaller and vacuolated and finally disappearing. The capillaries are narrowed. The centrum is more solid or may be cystic. By hyaline degeneration the corpus albicans is formed. This stage extends over several months.

The Corpus Luteum of Pregnancy.—According to Meyer, from the second month of pregnancy on, there is a coarser and cruder appearance of the corpus luteum owing to the epithelial cells becoming larger and assuming more irregular forms as well as harboring larger fat droplets, and the capillaries are covered by more fibrils and spindle cells, and therefore have a thicker wall, and finally, the lutein cells are surrounded by a much fibrillated connective tissue rich in spindle cells. This strong connective tissue framework and the well preserved lutein epithelium in the period from the third to the fifth month form a contrast to the corpus luteum of menstruation. Likewise the hyaline degeneration is long delayed. The thecal cells are retained sometimes up to the fourth month but are insignificant and decrease in number. Cysts may be found in the corpus luteum of pregnancy with the epithelium nevertheless well preserved. In the final month of pregnancy the thecal cells flourish again. According to Aschoff,[46] there are only two developmental periods in the corpus graviditatis: a period of growth and a period of retrogression. The period of growth is prolonged and leads to a much larger body. There are no hemorrhages; hence there is a

46. Aschoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924.

paler color. The total time of metamorphosis of the corpus graviditatis is about the same as that of the corpus menstruationis.

The Lutein Cell.—According to Robin, the characteristic cell of the corpus luteum was first described by Renaud in 1845, by Coste [31] in 1847, then by Dalton [32] in 1851. It is now almost universally held that the large epithelioid cells of the corpus luteum are hypertrophied granulosa cells continued from the graafian follicle. In some species of animals it has been necessary to distinguish "granulosa lutein cells" from the "theca lutein cells" because some of the theca cells persist in the corpus luteum as special elements, or they may be indistinguishable from the granulosa derivatives. In the human being this occurs to only a very small degree or more probably not at all; therefore the lutein cell is assumed to be the granulosa derivative. Although a few granulosa cells are expelled with the ova, most of them are retained and are transformed into lutein cells.

The lutein cell is rounded or polyhedral, or the shape may vary according to its environment. The large eccentric vesicular nucleus contains one prominent and one or two other nucleoli. The nucleus of the mature cell is nearly double that of the early form. An inner or perinuclear homogenous endoplasm may be distinguished from an outer exoplasm which is vacuolated. The Golgi apparatus appears as a large perinuclear network with wide meshes and relatively narrow trabeculae which are sometimes fragmented.

Typical mitochondria are easily demonstrated in fresh lutein tissue but they do not have any fixed morphologic relation to the fat droplets (Corner [27]). Chondriosome substance, i. e., mitochondria, is present in abundance in all developmental stages of the corpus luteum but is lacking during regression. It appears in the form of rods and granules lying free in the cell plasma, or it may be localized in the reticular system of the cell (Chydenius [47]). During the time when the mitochondria were most enthusiastically studied several observers attributed to them the ability to form fat in the cell. Concerning this, Aschoff [46] says: "After the work of Arnold and Altman, one becomes convinced more and more that the so-called chondriosomes play a part in the building up of the fat droplets; at any rate, there can be no doubt that when the fat first becomes visible it is in the form of very fine droplets which we also call microsomes or liposomes. Whether these really correspond to the chondriosomes is another question. Today one inclines to the view that the building up of fat within the cells is a more diffuse process."

Many of the controversies concerning the finer structure of the lutein cell may be explained by the wide variation in the fixing and staining methods used, as well as by the different sources of the material

47. Chydenius, J.: Arb. a. d. path. Inst. zu Helsingfors **4**:319, 1926.

studied. Chydenius,[47] using different histologic and histochemical preparations of identical or similar specimens, has succeeded admirably in clarifying many of the apparent disagreements occurring in the literature. It would be difficult to condense this extensive comparative study without losing much of its force.

PHYSIOLOGY

Formerly studies of the corpus luteum were largely anatomic, and it may be said that an anatomic study was a goal in itself. Nowadays the tide has turned, and the physiology is dominant. Although anatomy is still an important fundamental science, the modern scientific standpoint does not limit itself to the demonstration of the anatomic factors alone but requires that data be classified and arranged according to the cycle of development and interpreted from the standpoint of physiology. Likewise, any consideration of pathology must embrace both anatomy and physiology. The distinction between these three fundamentals cannot always be sharply made.

The following consideration of the physiology of the corpus luteum is more or less arbitrarily divided into: (1) that of the correlation of ovulation, phase of corpus luteum development and stage of the menstrual cycle, (2) that of the chemistry of the corpus luteum and (3) that of the function of the corpus luteum.

Since the process of ovulation has been mentioned and the evolution of the corpus luteum described, their relation to the stages in the menstrual cycle may now be considered.

Correlation of Ovulation, the Phase of Development of the Corpus Luteum and the Stage of the Menstrual Cycle.—A time and causal relation between ovulation, developmental phase of the corpus luteum and menstruation is now generally accepted. Ovulation is placed at approximately the middle of the intermenstrual period. The development of the corpus luteum is completed and regression has begun before the onset of menstruation. However, certain exceptions will be noted. These correlations are derived from anatomic and physiologic studies as well as from clinical records.

The present concept is the end-result of an evolutionary process in thought extending over more than a century. Thus Home [21] maintained that menstruation was totally unconnected with the formation of ova, with their leaving the ovary or with their impregnation. It should be noted, however, that he wrote at a time near the close of the controversy as to whether the corpus luteum was ever found without impregnation. Négrier [22] was led to believe from one observation that the menstrual hemorrhage is at its highest point when the rupture of the

vesicle occurs. Then Pouchet,[48] reviewing the literature for centuries before his time and adding his own observations, deduced by very excellent logic that, in the whole animal series the ovary expels its ova independently of fecundation; that the ova are expelled at a fixed time and in relation to the periodic superexcitation of the sexual organs; that graafian vesicles in women expel their ova only at the time of menstruation either immediately after or one, two, three or even four days later, and that therefore it is easy to determine accurately the intermenstrual time when conception is physically impossible and the time when there is some possibility of it.

During the next half century Pouchet's assertion that ovulation and menstruation coincide was generally accepted. However, some exceptions were noted and were not satisfactorily explained. Inferences from animal experiments were misleading owing to the failure to differentiate between the estrous cycle of animals and the human menstrual cycle. Conclusions drawn from material obtained at autopsy were also open to criticism because of the lack of data concerning the last menstruation and because of the influence of the causes of death on the condition of the ovary. With improvement in operative technic a new line of investigation was developed which led to the accumulation of conflicting data. Thus Leopold and Mironoff [49] observed the ovaries of forty-two patients whose menstruation had occurred from three to thirty days before operation. They found thirty corpora lutea corresponding to the last menstruation, but there were many exceptions to the coincidence of ovulation and menstruation. The true significance of these observations was apparently not appreciated by them, for they repeatedly mentioned that ovulation was produced by menstrual congestion.

Tait [50] in forty-nine operations found ovulation corresponding to menstruation only nine times, while Arnold [51] with fifty-four cases reported correspondence in thirty-nine. As late as 1908 Schaeffer [52] stated that in the majority of cases rupture of the follicle occurs at the same time as menstruation even though he had read and criticized Fraenkel's case reports published five years earlier showing that ovulation occurred in the midmenstrual period.

48. Pouchet, F. A.: Théorie positive de l'ovulation spontanée et de la fécondation des mammifères et de l'espèce humaine, basée sur l'observation de toute la série animale, Paris, J.-B. Baillière, 1847.

49. Leopold, G., and Mironoff, M.: Arch. f. Gynäk. **45**: 506, 1893-1894.

50. Tait, L.: Lancet **2**:1044, 1888.

51. Arnold, G.: Ueber das zeitliche Verhältnis der Ovulation zur menstruellen Blutung, Würzburg, Stahel, 1887.

52. Schaeffer, R., in Veit, J., and Stoeckel, W.: Handbuch der Gynäkologie, ed. 2, Munich, J. F. Bergmann, 1908, vol. 3, pt. 1, p. 3.

Since 1900 there has developed an ever increasing interest in the time of ovulation, particularly in its relation to sterility and to the "safe period" for birth control. Much of the accumulated evidence is circumstantial and inaccurate, and contains several variables making the drawing of conclusions a difficult and questionable procedure. The most abundant evidence bearing on this subject comes from two sources: records of pregnancy resulting from isolated intercourse and observations of ovaries at laparotomies. The first class of evidence depends on the honesty and accuracy of untrained observers in wide variety and unwarrantedly assumes knowledge of the duration of the viability and potency of spermatozoa. The second, while contributed by trained observers, depends on statements of untrained observers as to the time and regularity of menstruation. Furthermore, the exclusion of the influence of pathologic conditions is accomplished with varying degrees of success. At times part of this difficulty has been obviated by studying histologic sections of removed corpora together with preparations of endometrium taken at the same time.

A significant procedure of Seitz and Wintz showed that castration by means of x-ray before the fourteenth day resulted in cessation of the menses in 95 per cent of the cases, while castration after the fourteenth day did not influence the next period in 96 per cent. Comparable to this is the disturbance in menstrual rhythm produced by removing corpora lutea at varying times in the cycle.

Studies of the age of very young embryos as related to the time of ovulation are too few, and the results show too wide a "scatter" to be helpful in this problem.

Vaginal smears, which are such accurate indicators of the stages of the estrous cycle in animals, are not significant with respect to the human being, for they do not show characteristic cyclic changes.

The estrogenic hormonal content of the blood is assumed by Frank and Goldberger [53] and by Siebke [54] to place ovulation in the midmenstrual period, but the amount of hormone in the blood samples is so small that no sufficient number of test animals could be used to make this test reliable.

The variation in the amplitude and rate of contraction of the fallopian tube, which has been shown by Seckinger and Snyder [55] to be greatest just after ovulation—a finding confirmed by fluoroscopic studies of tubes into which iodized poppy-seed oil 40 per cent had been injected gives promise of furnishing important information. Their

53. Frank, R. T., and Goldberger, M. A.: J. A. M. A. **86**:1686, 1926.

54. Siebke, H.: Arch. f. Gynäk. **146**:417, 1931.

55. Seckinger, D. L., and Snyder, F. F.: Bull. Johns Hopkins Hosp. **39**:371, 1926.

observations on the cyclic alterations of the epithelium of the tubes is confirmatory evidence.

Midmenstrual pain in the pelvis was attributed to the process of ovulation by Priestley [56] in 1872. He found no record of a similar observation in the literature. Like many subsequent observers, he probably dealt with endometriosis or an inflammatory condition. Instances of midmenstrual pain *(Mittelschmertz)* have since been multiplied many times. Recently, Pratt [57] correlated symptoms related to the region of an ovary with evidence at operation of a newly ruptured follicle. Records kept by normal women are cited showing discomfort in one or the other ovary just two weeks before the succeeding menstruation. Occasional pelvic examinations at these times demonstrated enlargement and tenderness of the corresponding ovary. The accumulated data on midmenstrual phenomena are extremely suggestive and possibly confirmatory as to the time of ovulation.

Since the process of ovulation has never been observed in the human being, proof of its occurrence seems to lie in the recovery of an ovum just after its extrusion from the follicle. This has been accomplished by Allen, Pratt, Newell and Bland,[58] who recovered five ova from the fallopian tubes between the fourteenth and the sixteenth day. Study of these ova in fresh and in fixed sections showed that degeneration takes place rapidly. By correlating the state of development of the corpora lutea with the condition of the ova an attempt was made to compute the time of ovulation. This was estimated as between the twelfth and fourteenth days of the menstrual cycle. The conclusions drawn from these data were criticized by Evans and Swezy,[59] who maintained that if only five ova were recovered in ninety cases studied the number is relatively too small to be conclusive. It seems, however, that the latter workers were not then fully conversant with the difficulties in obtaining ideally planned human material, which is so easy in experimentation with animals. Human material must be studied as chance offers; it can not be approached in the manner of a carefully arranged experiment.

While menstruation without ovulation has been described in the human being, adequate reports are still infrequent. Furthermore, the corpus luteum is not concerned in this type of menstruation, and the matter is therefore outside the province of this review.

The literature bearing on the "safe period" as applicable to birth control was well reviewed by Dickinson,[60] who concluded that there is

56. Priestley, W. O.: Brit. M. J. **2**:431, 1872.

57. Pratt, J. P.: Am. J. Obst. & Gynec. **27**:816, 1934.

58. Allen, E.; Pratt, J. P.; Newell, Q. U., and Bland, L. J.: Human Tubal Ova; Related Early Corpora Lutea and Uterine Tubes, Contrib. Embryol. no. 414, Carnegie Institution, Washington, 1930.

59. Evans, H. M., and Swezy, O.: Am. J. Physiol. **96**:628, 1931.

60. Dickinson, R. L.: Am. J. Obst. & Gynec. **14**:718, 1927.

no time in the month at which conception has not occurred, "but the height of fertility belongs to the week or ten days following menstruation."

A striking difference in the interpretation of the same data is shown if one compares Schröder's [61] report of 898 cases in which there was a regular four week cycle and in which the endometrium in each case was studied with Evans and Swezy's interpretation of his data. Schröder concluded that there is a consistency in the uterus-ovarian relationship that cannot be questioned and that ovulation occurs in every cycle since functional corpora lutea were present in nearly half of his cases, the other cases showing the preovulation stage of the endometrium. Evans and Swezy, on the other hand, concluded that no data compiled justify one in assigning ovulation to an invariable period of the menstrual cycle even though the period is broadly defined. Furthermore, utilizing Schröder's material differently, they concluded that ovulation, while more apt to occur in the midmenstrual period, occurs at all times in the human menstrual cycle.

In view of all the foregoing evidence it seems justifiable to say that there is usually a definite time relation between ovulation and menstruation. The causal relation is strongly suggested but not conclusively proved.

CHEMISTRY

The demonstration that the ovaries influence the uterus by way of the blood stream stimulated numerous investigations, among which were many of a chemical nature. The striking appearance of the corpus luteum attracted special attention to it. The early preparations, such as desiccated and fresh gland substance and saline extracts, were too crude to be evaluated chemically. The first products capable of chemical interpretation were the lipoids, and the introduction of lipoid solvents produced the first potent products. The pigments are of interest, not on account of any potency, but because they are the conspicuous elements which caused the corpus luteum to be so named. While theelin and progestin have been isolated from the corpus luteum, the former has a wide distribution and therefore cannot be considered characteristic of the corpus luteum. On the other hand, progestin has been shown to be a particular product of the corpus luteum in some species of animals. It also, however, has been demonstrated from other sources.

So many important discoveries have originated from experimentation with animals that a clear concept of the status of the human corpus luteum cannot be obtained if all the work on animals is excluded. Therefore, such work of this type as seems pertinent will be mentioned.

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61. Schröder, R., in Veit, J., and Stoeckel, W.: Handbuch der Gynäkologie, ed. 3, Munich, J. F. Bergmann, 1928, vol. 1, pt. 2.

For simplification, discussion of the chemistry will deal with the following subjects separately: theelin, progestin, lipoids and pigments.

Theelin.—The events leading up to the isolation and concentration of theelin will be briefly reviewed in the section on function. For a more detailed account I may refer the reader to the publications of Allen and Doisy [62] and Frank.[63] Research on the chemical nature of theelin dates from the discovery of an accurate method of testing the hormone by Allen and Doisy [64] in 1923. They were able to show by the now well known vaginal smear test that an alcoholic extract of the ovaries is capable of inducing all the stages of estrus in castrated animals, thus obtaining absolute evidence of the activity of a stable extract. From this time on rapid advances were made in the chemical examination of the compound.

Unfortunately, alcohol and volatile solvents extract from the tissues a complex group of substances with very similar properties, and consequently for a while little progress was made in the determination of the exact chemical nature of the hormone. The limitation of material was also embarrassing. Nevertheless, this extract was soon proved to contain carbon, hydrogen and oxygen and to be free from nitrogen, but separation from contaminants was difficult.

The next great advance was the discovery by Aschheim and Zondek,[65] in 1927, that an apparently identical hormone is excreted in the urine of pregnant women. This greatly increased the amount of material available for study, for a simple benzene extract of the urine was found to yield large quantities of the hormone in a state of purity which formerly was almost unattainable. Great interest was aroused, and groups of investigators all over the world began to study the chemical properties, and in a relatively short time the hormone was obtained in crystalline form.

The compound was obtained independently in pure crystalline form by different groups of investigators. Doisy had priority in publication, as his original communication was made to the Thirteenth International Physiological Congress in August 1929, and the detailed account of his work was published in the following year.[66] In October following Doisy's report, Butenandt announced his success.[67] Their work has now been amply confirmed by others.

62. Allen, E., and Doisy, E. A.: Physiol. Rev. **7**:600, 1927.

63. Frank, R. T.: Female Sex Hormone, Springfield, Ill., Charles C. Thomas, Publisher, 1929.

64. Allen, E., and Doisy, E. A.: J. A. M. A. **81**:819, 1923.

65. Aschheim, S., and Zondek, B.: Klin. Wchnschr. **6**:1322, 1927.

66. Doisy, E. A.; Thayer, S., and Veler, C. D.: J. Biol. Chem. **86**:499, 1930.

67. Butenandt, A.: Naturwissenschaften **17**:878, 1929.

Extensive work has been done with reference to the structural formula and properties of theelin, which need not be recorded here. Doisy [68] characterized it as a ketomonatomic alcohol having a molecular weight of 270 and a formula of $C_{18}H_{22}O_2$ with the hydroxyl phenolic in character.

The nomenclature is confusing, many different terms having been applied to the same substance. "Theelin" has been used here because it is the term applied by Doisy, the first to announce it in pure crystalline form. The following synonyms are found in the literature: "female sex hormone," "α-hormone," "α-follicular hormone," "ovarian follicular hormone," "feminin," "folliculin," "menformon," "estrin," "dioxyestrin," "ketohydroxyestrin," "thelykinin," "estrone," and "3-hydroxy-17-keto-1,3,5,estratiene." At least forty different trade names are in existence. The potency of these trade products vary from approximately zero to a thousand or more rat units per cubic centimenter. This variability in potency leads to skepticism with reference to some of the brilliant therapeutic results reported.

A comparison of the reports of potency of various products is difficult, for there is great variation according to the test animal used as well as to the number and frequency of the doses given. Some reports are in terms of mouse units and others are in terms of rat units, but fortunately there is now an increasing tendency to accept the international unit as standard.

Dodds [69] in 1934 published some interesting observations on the chemical relationships of estrin. He assigned it to a group known as the condensed carbon ring compounds, consisting of a series of three six membered rings fused together with a five member ring at one end of the molecule. It is a derivative of phenanthrene, which formerly was not known to occur in the animal organization, although for many years the phenanthrene molecule has been known to be possessed by certain drugs.

The sterols or condensed carbon ring compounds are now known to be of very great importance among the organic compounds occurring in the body. They include cholesterol and its derivatives the bile acids, ergosterol, calciferol (vitamin D) and the sex hormones. Dodds suggested the possibility that various polar groups, side chains, double bonds, etc., are associated with the different types of activity of the different molecules. In general, the greater the nonsaturation of the sterols the greater is their activity. If theelin is partially dehydrogenated and two atoms of hydrogen are added, a compound known as

68. Doisy, in Allen, E.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1932.

69. Dodds, E. C.: Lancet **1**:931, 987 and 1048, 1934.

dihydroxyestrin is produced which is much more active than theelin. Calciferol (vitamin D) produced full estrus in 20 per cent of ovariectomized animals treated.

Theelol, which has one more molecule of water than theelin, is an alcoholic derivative of theelin and has the formula $C_{18}H_{24}O_3$, was crystallized by Marrian [70] in 1930. It differs from theelin in that it is effective when administered orally and is less active than theelin. "Theelol" likewise has several synonyms: "trihydroxyestrin," "emminin," "follicular hormone hydrate" and "3,16,17-trihydroxy-1,35-estratiene (estriol)." Theelol can be converted into theelin. Theelin and theelol have not been obtained in crystalline form from the corpus luteum, but by other means these compounds have been shown not to vary in their reaction when taken from different sources, and therefore general statements about theelin apply equally well to the estrogenic substance obtained from corpora lutea.

By oxidation of sterols and bile acids Ruzicka and his collaborators [71] have obtained a preparation of the testis hormone (androsterone) from urine. It was found to be effective in producing comb growth in capons. They have shown a close relationship in structure between theelin and the synthesized sex hormone.

Progestin.—The isolation and crystallization of progestin were tantalizing and elusive until 1934. A crystalline fraction of an extract of the corpora lutea of hogs was announced in 1931 by Fels in several brief reports,[72] but no data were presented to indicate that the substance was chemically pure. Allen [73] in 1932 announced the preparation of crystals, which he later found were not chemically pure. Fevold and Hisaw [74] in 1932 described crystals readily soluble in alcohol, ether acetone and other similar solvents but much less soluble in water, which did not prove to be chemically pure.

During the year 1934 success came to different groups working independently, but Butenandt gained priority when he reported before the Deutsche Gesellschaft für innere Medizin on April 11. Details of the report were published in July.[75] He obtained physiologically active material in crystal form to which he assigned the formulas $C_{21}H_{30}O_2$

70. Marrian, G. F.: Biochem. J. **24**:1021, 1930.

71. Ruzicka; Goldberg and Brüngger: Helvet. chim. acta **17**:1389 and 1935, 1934.

72. (*a*) Zentralbl. f. Gynäk. **55**:2765, 1931; (*b*) Berl. klin. Wchnschr. **10**:1639, 1931; (*c*) Arch. f. Gynäk. **144**:280, 1931.

73. Allen, W. M.: J. Biol. Chem. **98**:591, 1932.

74. Fevold, H. L., and Hisaw, F. L.: Proc. Soc. Exper. Biol. & Med. **29**:620, 1932. Fevold, H. L.; Hisaw, F. L., and Leonard, S. L.: J. Am. Chem. Soc. **54**: 254, 1932.

75. Butenandt, A.: Wien. klin. Wchnschr. **47**:934, 1934.

or $C_{20}H_{28}O_2$. A few days later a publication by him [76] established the formula $C_{21}H_{30}O_2$. In the same month of August this result was also announced by Allen and Wintersteiner [77] and Fels and his co-workers.[78] The latter named their products "luteosteron A, B, C and D," which here will be referred to as fractions A, B, C and D. They reported on "the preparation of the pure hormone from the corpus luteum." [79] Their article contains a brief summary of the properties of the crystalline products, followed by an argument that the authors were the first to isolate the pure hormone rather than Butenandt and his co-workers. Later Slotta and his associates [80] stated that the tetracyclic ring system of the sterols had been proved, with a carbonyl in position 3 and an acetyl in position 17. They considered that the difference between fractions C and D is due to their being isomers. Butenandt and his collaborators [81] presented details previously reported in abstract form only. They considered it a diketone. Its evaluation by means of infantile rabbits was described and a unit of activity proposed.

Wintersteiner and Allen [82] in October 1934 reported the isolation of several crystalline compounds from the active extracts of hog corpora lutea. Their methods and results were described in detail. Their fractions A and D are inactive, but B and C are active. Fraction B has the formula $C_{21}H_{30}O_2$ and reacts like progestin. It contains both oxygen atoms and a carbonyl group. Its melting point is 128 C. Other crystalline products of equal potency, but of different crystal form and melting at 120-121 C., were also obtained which they think either represent a polymorphous modification of the hormone or are mixed crystals containing a small amount of contaminant. The main constituent of the crude crystalline fractions is an inactive compound of the probable composition $C_{21}H_{34}O_2$. Fractions C and D of Fels and Slotta apparently correspond to fractions B and C of Wintersteiner and Allen.

The possibility of a synthesis of the estrogenic substances has been suggested by the recent work of Butenandt and his associates [83] on the decomposition of stigmasterol to a substance having the activity of

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76. Butenandt, A., and Westphal, U.: Ber. deutsch. chem. Gesellsch. **67**:1440, 1934.

77. Allen, W. M., and Wintersteiner, O.: Science **80**:190, 1934.

78. Fels, E.; Slotta, K. H., and Ruschig, H.: Klin. Wchnschr. **13**:1207, 1934.

79. Slotta, K. H.; Ruschig, H., and Fels, E.: Ber. d. deutsch. chem. Gesellsch. **67**:1624, 1934.

80. Slotta, K. H.; Ruschig, H., and Blanks, E.: Ber. d. deutsch. chem. Gesellsch. **67**:1947, 1934.

81. Butenandt, A.; Westphal, U., and Hohlweg, W.: Ztschr. f. physiol. Chem. **227**:84, 1934.

82. Wintersteiner, O., and Allen, W. M.: J. Biol. Chem. **107**:321, 1934.

83. Butenandt, A.; Westphal, U., and Colber, H.: Ber. d. deutsch. Chem. Gesellsch. **67**:1611, 1934.

corpus luteum in doses only slightly greater than those of the pure hormone. Also Fernholz [84] in a preliminary communication reported that he had prepared from the stigmasterol of soya beans a pure diketone which appears to be identical with the Fels and Slotta fraction C.

For a further detailed study of the sex hormones and the allied compounds the reader is referred to the excellent reviews of the subject by Dodds [69] and Cook.[85]

The corpus luteum hormone has been found in the placenta and the urine of pregnant women in rather small quantities (Adler and others [86] and Ehrhardt [87]). Ehrhardt found it in the amount of 10 rabbit units in the mature human placenta, and Fels also obtained it in the human placenta and urine.

Previous attempts at isolation of progestin from the placenta and urine of pregnant women by Philipp,[88] Fels,[89] Allen,[73] Clauberg [90] and Ehrhardt and Weigel [91] were unsuccessful.

Although most of the work just cited was done on animals it opens a fascinating chapter which suggests most interesting future possibilities. How it will be applied to the human problem is not yet clear. It seems important to present it here for consideration and speculation, but conclusions are hardly permissible.

The presence of progestin in the human corpus luteum has frequently been inferred on theoretical grounds. Clauberg, Thiel and Ziecker [92] obtained a questionable reaction with 50 Gm. of human corpora lutea and speculated that there might be a demonstrable amount of luteal hormone if 70 Gm. of material were used. In 1934 a group of workers [93] collected human corpora lutea for assay. Human and hog corpora lutea were extracted in the same manner and at the same time with the result that the extract of hog corpora lutea induced an estrual reaction at 20 ± 5 Gm. while that of human corpora lutea up to 40 Gm. did not induce estrus.[93] In February 1935, another assay of human material was completed by the same group (unpublished). With the immature rabbit as a test animal, progestin was found to be present in 100 Gm. of human material. Evidently, progestin is much

84. Fernholz, E.: Ber. deutsch. chem. Gesellsch. **67**:1855, 1934.

85. Cook, J. W.: Nature, London **134**:758, 1934.

86. Adler, A. A.; de Fremery, P., and Tausk, M.: Nature, London **133**: 293, 1934.

87. Ehrhardt, K.: München. med. Wchnschr. **81**:869, 1934.

88. Philipp, E.: Zentralbl. f. Gynäk. **54**:2754, 1930.

89. Fels, E.: Arch. f. Gynäk. **158**:364, 1934.

90. Clauberg, C.: Die weiblichen Sexualhormone, Berlin, Julius Springer, 1933.

91. Ehrhardt, K., and Weigel, W.: Endokrinologie **13**:225, 1933.

92. Clauberg, C.; Thiel, H. W., and Ziecker, R.: Arch. f. Gynäk. **152**:61, 1932.

93. Pratt, J. P.: Endocrinology **18**:667, 1934.

less abundant in human than in hog corpora lutea. Also, it may be elaborated elsewhere in the ovary or, as has been shown, in the placenta.

Lipoids and Fats.—For years the study of the chemistry of the corpus luteum centered around the lipoids and fats because they were thought to be or to contain the active principles of the gland. A great deal of study was devoted to determining the kinds and amounts of these substances present at different times and in varying conditions. When the estrogenic substances were isolated and freed from the lipoids, interest was diverted into other channels, and the lipoids have received less attention during the last few years. In spite of the extensive study of the lipoids, they have not been demonstrated to be potent agents; however, they are associated with the principles proved to be active.

With the introduction of osmic acid into histologic technic, structures were demonstrated in the corpus luteum which in the nineteenth century were usually designated simply as fat and were considered as an expression of a degenerative process. The same interpretation is acceptable today. However, in the interim wide excursions have been made in the methods of study as well as in the interpretation of the facts observed.

The methods used may be grouped as histochemical, physical (polarized light) and chemical. A moment of reflection will enable one to enumerate a long list of variables which these methods individually and collectively may introduce. In spite of the controversies which have arisen some fairly definite conclusions have been reached.

Chydenius,[47] who made an excellent comparative study of most of the work on lipoids and investigated the subject thoroughly himself, stated that one is not justified in concluding in respect to the corpus luteum that a high function of the cells springs from richness in lipoids, and that studies of the lipoids, in spite of the attempt to distinguish the various kinds of lipoids, can easily lead to an unjustifiable conclusion if there is not simultaneous careful consideration of the results of other histologic methods of investigation. He appended to his article a bibliography which contains the names of all the important workers in the field.

Hermstein[94] analyzed human corpora lutea of different ages and found that the total lipoid varied from 5.9 to 11.9 per cent. Cholesterol, cholesterol esters and phosphatides are most abundant at the time of full development, while after the onset of menstruation neutral fats, fatty acids and soaps increase relatively. Phosphatides are abundant in all phases.

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94. Hermstein, A.: Arch. f. Gynäk. **124**:739, 1925.

Bertoloni [95] divided his material arbitrarily according to the time in the cycle: first to eighth, ninth to fourteenth, fifteenth to twenty-first, twenty-second to twenty-eighth, twenty-ninth to forty-second day of the cycle. His results, shown in the accompanying table, are in accord with those in most other reports. He admits that staining methods are not altogether reassuring, and that too many doubts remain to permit drawing definite conclusions. The table shows that he was in accord with Miller,[38] who said that in the corpus luteum of pregnancy scarcely any lipoids are demonstrable. It is generally conceded that lipoids are most abundant during the regression of the corpus luteum as well as in atresia of the follicle. No particular significance has been attached to the relative amounts of the different kinds of lipoids.

Interest in the extensive investigation of the lipoids has been largely academic. However, this line of research eventually led to the extraction of the really potent products of the ovary. When attention became focused on the function of the corpus luteum it was entirely logical to pursue the study of the reactions of any demonstrable fraction of that structure with the hope that the source of the function might be discovered. Since recent work has shown the close association of pure sex hormone with cholesterol and perhaps its derivation from that lipoid, the latter may again come into prominence.

Pigments.—Little work has been done on the pigments of the human corpus luteum, but inferences are drawn from experiments on animals. Asdell [96] noted that pigmentation of the corpus luteum follows the general coloration of the body fat. Pigment is accordingly found in the human being in whom the body fat is also pigmented. In animals like the rabbit in which the body fat is not colored the corpus luteum is likewise unpigmented. Asdell [96] further noted that since the occurrence of the pigment is confined to a few species and is dependent on the occurrence of pigment in the body fat, it is unlikely that any functional purpose is to be ascribed to it. He cited the work of Palmer and Eckles, who demonstrated the close relationship between the body pigmentation and the coloring matter of the feed.

Thudicum [97] in 1869 pointed out the close relationship between the pigments of the corpus luteum and the yellow pigments generally in plants and animals. Also he criticized earlier workers who confused the ovarian pigment with hematoidin or with other derivatives of hemoglobin or of bilirubin. Escher [98] in 1913 found the pigment of the corpora lutea of cows identical with carotene ($C_{40}H_{56}$) from carrots as

95. Bertoloni, G.: Folia gynaec. **21**:645, 1925-1926.
96. Asdell, S. A.: Physiol. Rev. **8**:313, 1928.
97. Thudicum, J. L. W.: Proc. Roy. Soc., London **17**:253, 1869
98. Escher, H. H.: Ztschr. f. physiol. Chem. **83**:198, 1913.

Bertolini's Results in the Study of the Lipoids of the Corpora Lutea

Lipoid	Follicle		Follicle in Process of Atresia		Atretic Follicles	Formation and Regression of the Corpus Luteum										Corpus Luteum of Pregnancy			
						1-8th Day		9-14th Day		15th-21st Day		22d-28th Day		29th-42d Day					
	Membrana Granulosa	Theca	Membrana Granulosa	Theca	Theca	Membrana Granulosa	Theca	Membrana Granulosa	Theca	Membrana Granulosa	Theca	Membrana Granulosa	Theca	Membrana Granulosa	Theca	Membrana Granulosa	Theca	Membrana Granulosa	Theca
Phosphatide cerebrosides	+	+++	++	+++	++++	+	+++	+++	++++	++	++++	+++	++++	++++	++++	....	....	+	....
Cholesterol cephalin	..	.....	++	+++	++++	..	.....	.....	.....	....	.....	+++	.....	++++	++	++	....	....	....
Cholesterol glycerin	..	.....	....	+++	++++	..	.....	.....	.....	....	.....	+++	.....	++++	++	....	....	....	....
Cholesterol fatty acid	..	.....	....	+++	++++	..	.....	.....	.....	....	.....	++	.....	++++	....	....	....	....	....
Cholesterol ester, fatty acid	..	.....	....	+++	++++	..	.....	.....	.....	....	.....	+++	.....	++++	....	....	....	....	....
Neutral fats	..	.....	....	++++	++++	..	.....	.....	.....	....	.....	++++	.....	++++	....	+	....	....	....

to melting point, percental composition and spectroscopic aspects. He also mentioned the theory that pigments of this type may function in connection with cell respiration, for they readily combine with oxygen.

Since pigment in the corpus luteum has not been shown to have any particular relation to the sex hormones, it does not require further attention here. It should be mentioned in passing that blood pigment in the corpus luteum has frequently been mistaken for the true pigment.

FUNCTION

Compilation of all the ideas concerning the function of the corpus luteum would be onerous and useless. Some of the ideas are founded on fact, some on fancy, and some have no foundation but are covered by that captivating term "empirical." In all the vast literature on the subject, however, there is discernible a trend of thought to the present concept, which by no means can be considered final. Interpretation of the nature and manner of function of the corpus luteum experienced a rather sharp change at the beginning of the present century. Therefore, the subject may be considered in two divisions—the first consisting of heterogeneous material, and the second, of material relating chiefly to the possibility that the structure is a gland of internal secretion.

What the early writers on the corpus luteum thought of its function is rather hazy. De Graaf [10] in 1688 thought the corpus was in some way related to coition. Malpighi [11] in 1687 believed that it gave rise to the follicle and the ovum. The association of the corpus luteum with pregnancy was established early but its occurrence outside of pregnancy was debatable until nearly the middle of the last century.

Home [21] in 1817-1819 partly corroborated Malpighi's opinion, for he thought the corpora lutea were glandular structures in which the ova were produced. Meckel [15] in 1825 also believed they were glandular and secreted the female seminal fluid. This indefinite concept of the corpus luteum as a glandular structure was shared by many.

Négrier [22] in 1840 considered the yellow material as food destined to support the life of the germ after its separation. He expressed the opinion that this would be a first placenta and that the bed hollowed out in the ovarian parenchyma would be its first uterus.

Just when the idea originated that the corpus luteum serves especially to heal the ruptured follicle with a minimal scar is difficult to determine. Clark [26] in 1899 stated that its function was usually passed over without remark or the investigator frankly stated that nothing was known concerning it. Clark asserted further that the theory most usually advanced was that the corpus luteum by its rapid growth fills out the empty follicle and thus restores the equilibrium of the circulation of the ovary. He cited some of his own observations to substantiate this theory.

Beard [99] in 1897 suggested that the deciduum is prepared through the function of the corpus luteum, and that degeneration of the corpus luteum at the end of the menstrual cycle results in abortion just as degeneration of the corpus luteum before the end of gestation results in parturition. This abortion makes possible the resumption of ovulation, which has been held in check during the normal functioning of the corpus luteum.

The second phase of interpretation of the function of the corpus luteum began just at the close of the last century when its intriguing appearance suggested that it be considered as a gland of internal secretion. The publication of Prenant's [3] article in 1898 and the announcement by von Born stimulated great interest in this new theory. Fraenkel [19] in 1903 emphasized the importance of the corpus luteum as a cause of menstruation and ascribed to it a specific action in the nidation of the ovum. Because suppuration and adhesions occurred in the majority of Fraenkel's experimental animals, his results were criticized by Daels.[100]

Possibly influenced by Baumann's success with thyroid preparations for hypothyroidism, Landau [101] in 1896 tried desiccated ovary to alleviate the symptoms in women after ovariectomy, but met with indifferent success. Removal of the ovaries with subsequent transplantation to another site by Knauer [102] in 1900 and Halban [103] demonstrated that the ovaries influenced the uterus by means of an internal secretion carried through the blood. From that time various extracts of the ovary and its parts were prepared and used both experimentally and clinically. Fraenkel [19] in 1903 was influential in focusing attention on the corpus luteum as the most likely source of an internal secretion. His hypothesis was so plausible that the extensive use of some form of corpus luteum preparation was soon established and has not yet been dislodged. The wave of enthusiasm for this form of therapy increased during the next decade.

Frank [104] in 1910 wrote:

> Our knowledge relating to the function of the corpus luteum is limited to a few isolated and disjointed facts. The minority are based on reliable experimental data; the majority are insecurely founded on clinical observations, which permit of widely differing interpretations.
>
> Even a cursory study of the corpus luteum under the microscope will convince an unbiased observer that a structure which is stamped with the hall-marks of an extremely active gland must possess at least some, and probably important functions.

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99. Beard, John: The Span of Gestation and the Cause of Birth, Jena, Gustav Fischer, 1897.
100. Daels, F.: Surg., Gynec. & Obst. **6**:153, 1908.
101. Landau, L.: Berl. klin. Wchnschr. **33**:557, 1896.
102. Knauer, E.: Arch. f. Gynäk. **60**:322, 1900.
103. Halban, J.: Monatschr. f. Geburtsh. u. Gynäk. **12**:496, 1900.
104. Frank, R. T.: Arch. Int. Med. **6**:314, 1910.

With experimental injections of corpus luteum extract he obtained generally unsatisfactory results. In regard to ovotherapy he wrote: "The results obtained have shown great variations, seemingly depending about equally on the subjective impressions of the physician and of the patient."

Until 1912 all the extracts made from various portions of the ovary were aqueous solutions, occasionally with the addition of weak alkalis or acids or of sodium chloride. In a series of experiments from 1908 to 1914 Iscovesco [105] produced a lipoid extract from the whole ovary and the corpus luteum and likewise from the placenta. These extracts were undoubtedly potent, as demonstrated by experiments on animals. Remarkable clinical results were also obtained by him, but these should be evaluated according to the statement of Frank quoted in the preceding paragraph. Some remarkable results are reported in the treatment of dysmenorrhea, amenorrhea, various conditions of hypo-ovarianism, menopause and senility. However, Iscovesco's work gave a new impetus to the study of the internal secretion of the ovary, the chief attention still being focused on the corpus luteum. Some question has been raised whether a pure product had been found or merely a greater concentration of the active substance.

The further progress in the purification and concentration of the prepotent factors derived from the ovary and placenta is a long and fascinating story, but since up to the present it concerns the human corpus luteum only by implication, one is not permitted to digress so far.

It should be noted, however, that Sonnenberg [106] in 1907 experimented with follicle fluid diluted with glycerin and failed to produce effects because he looked for results within thirty minutes. Adler [107] in 1912 extracted hashed ovaries and corpora lutea with distilled water and obtained a product which promoted growth in the uterus. He also found expressed juices active. Seitz, Wintz and Fingerhut [108] in 1914 prepared lipamin, a water-soluble product, which they believed to be one of the hormones of the ovary.

In 1914 Okintschitz [109] used glycerol mixed with saline solution to extract ovaries and corpora lutea of calves and pigs. In 1917 Frank [110] obtained fluid from the ovarian follicles of cows, which he injected into rabbits with the result that the uterus showed hyperplasia. Wintz [111] in

105. Iscovesco, M. H.: Rev. de gynéc. et de chir. abd. **22**:161, 1914.
106. Sonnenberg, N.: Berl. tierärtzl. Wchnschr. **39**:700, 1907.
107. Adler, L.: Arch. f. Gynäk. **95**:349, 1911.
108. Seitz, L.; Wintz, H., and Fingerhut, L.: München. med. Wchnschr. **61**: 1657 and 1734, 1914.
109. Okintschitz, L.: Arch. f. Gynäk. **102**:333, 1914.
110. Frank, R. T.: J. A. M. A. **78**:181, 1922.
111. Wintz, H.: Arch. f. Gynäk. **113**:457, 1920.

1920 also injected follicular fluid, but the number of injections was insufficient to induce more than rudimentary estrous conditions. Seaborn and Champy [112] in 1923 injected the follicular fluid of mares into rabbits and obtained in from eight to ten hours a congestion of the genital organs and a willingness to copulate. Attention to the follicles detracted from the corpus luteum as the sole or the most important source of the internal secretion of the ovary. The culminating event in the investigation of the follicles came with the discovery by Allen and Doisy of a means of standardizing and purifying the hormone from the follicular fluid. The material used was the follicle of the pig's ovary.

Although various early preparations from the corpus luteum were thought to be slightly potent, they are difficult to evaluate. Furthermore, in the light of present knowledge many of them could have had no activity at all, for it has been shown that the corpora lutea of some species of animals contain little or no follicular hormone. Allen and Doisy [113] in 1925, for the first time, satisfactorily isolated a hormone (theelin) from dated material of the human corpus luteum, thereby showing that the elaboration of a hormone by the human ovarian follicle is continued by its successor the corpus luteum. Later in 1925 Allen, Pratt and Doisy,[114] and subsequently Allen, Pratt, Newell and Bland,[115] showed the varying content of corpora lutea from different women and at different times in the sexual cycle. Zondek and Aschheim [116] in 1925 and 1926 obtained positive results from implants of corpora lutea.

The theelin content is greatest in young corpora, decreasing as the time of the next menstruation draws near. Considerable quantities are found during the period from the first to the third month of gestation and little or none at the end of gestation. At the end of gestation, therefore, the corpus luteum of woman does not share the responsibility for the large amounts of this hormone in the placenta, blood and urine.

Since theelin has a wide distribution in nature it cannot be considered specific for the corpus luteum. Therefore, the function of the corpus luteum on account of its elaboration of theelin is merely a continuation of the function of the follicle. The production of theelin is a function of the follicular apparatus as a whole and is not limited to one of its parts. Although theelin has been demonstrated to substitute

112. Seaborn, E., and Champy, C.: Compt. rend. Soc. de biol. **89**:1091, 1923.
113. Allen, E., and Doisy, E. A.: Proc. Soc. Exper. Biol. & Med. **22**:303, 1925.
114. Allen, E.; Pratt, J. P., and Doisy, E. A.: J. A. M. A. **85**:399, 1925.
115. Allen, E.; Pratt, J. P.; Newell, Q. U., and Bland, L. J.: Am. J. Physiol. **92**:127, 1930.
116. Zondek, B., and Aschheim, S.: Arch. f. Gynäk. **127**:250, 1925.

satisfactorily for ovarian function in spayed animals, a similar use has not yet been shown in the human being. Even the use of crystalline theelin has not greatly altered the situation.

The hypothesis proposed by Prenant, that the corpus luteum is a gland of internal secretion, has never been abandoned even though interest in it waned for a while when attention was focused on the follicle. In recent years it has come into the foreground again on account of the isolation of hormones from the corpus luteum of the sow. Such a hormone had long been predicated, so that its demonstration was preceded by a number of theories as to its nature and function.

Relaxin.—According to Hisaw,[68] "there are perhaps few phenomena that have the distinction of having been discovered and rediscovered by as many independent investigators as the extraordinary separation of the pelvic bones of the guinea pig during pregnancy. Duncan (1854) seems to have made the original observation and many have reported it since." Hisaw [117] found these pelvic changes to be under hormonal control. He found the relaxant substance in the blood serum of many mammals but not in the blood of cows or women. Later he found it abundantly in the placenta and corpora lutea of the sow and named it "relaxin." He observed that neither theelin nor relaxin alone could elicit the reaction, but that there was a synergistic relationship between the two hormones. He expressed the belief that theelin takes the major rôle during advanced pregnancy, while others suggest that in some species, at least, theelin is the sole factor. Most of the work with relaxin has been done with animals, but it is well known to obstetricians that many women show definite evidence of a widening of the symphysis and loosening of the sacro-iliac joints during pregnancy. Symptoms from this widening are not uncommon, and furthermore such widening has been demonstrated by x-ray photographs, but the evidence is too incomplete to justify any conclusions yet. Eymer [118] thinks relaxin is probably a follicular hormone.

Progestin.—The apparent potency of some of the earlier preparations from the corpus luteum can now be explained better by their theelin content than by any specific hormone. Herrmann [119] in 1915 apparently saw a progestational effect but overlooked it. Therefore, the discovery of a new hormone in the corpus luteum which seemed to have a specific action in some animals was emphatically welcomed and quickly applied by inference to explain many of the cyclic phenomena in the human

117. Hisaw, F. L.: (*a*) Proc. Soc. Exper. Biol. & Med. **23**:661, 1926; (*b*) Anat. Rec. **37**:126, 1927; (*c*) Physiol. Zool. **2**:59, 1929.

118. Eymer: Deutsche med. Wchnschr. **60**:27, 1934.

119. Herrmann, E.: Monatschr. f. Geburtsh. u. Gynäk. **41**:1, 1915.

being. Just as chemical studies leading to the isolation and crystallization of theelin were made possible by the discovery of Allen and Doisy,[64] so also the preparation of progestin in pure crystalline form has been made possible by the brilliant and informative experimental investigations of Corner and Allen.

Corner [120] in 1928 experimentally confirmed the earlier findings of Ancel and Bouin that the existence of progestational proliferation of the rabbit's uterus during the earliest days of pregnancy is dependent on the corpora lutea, and of Fraenkel that the corpora lutea are necessary to the implantation of embryos. Furthermore, Corner's experiments suggested the existence of a special action of the corpus luteum on the endometrium, bringing about progestational proliferation. By a modification of Herrmann's method, Corner and Allen [121] produced an alcoholic extract of corpora lutea of swine which induced the formation of progestational proliferation of the endometrium in the uterus of castrated rabbits. Subsequently, they [122] showed that normal implantation of embryos could be induced by these extracts.

The active extract of corpora lutea was named "progestin" by Allen.[123] Relaxin prepared by Hisaw and his co-workers also contained another substance which was later separated by them [74] and named "corporin." Clauberg,[124] using the method of Corner and Allen, called his product "lutin" and later "luteo-hormone." Wiesner [125] spoke of it as the "beta factor." Progestin, corporin, beta factor and luteal hormone are apparently identical.

The test animal for biologic assay is preferably the rabbit. Allen and Corner [122] arrived at the rabbit unit by injecting progestin into recently castrated adult female rabbits for five days, with autopsy on the sixth day. The least quantity that would produce progestational proliferation equal to that of a normal rabbit in a like period was considered as 1 rabbit unit. Clauberg used immature rabbits prepared by injection of theelin first. Noncastrated immature female rabbits were treated daily for eight days with injections of 10 mouse units of estrin before the injections of corpus luteum hormone were begun. Corpus luteum extract was then injected for five days and the animal killed the next day and examined for proliferation of the endometrium. The units obtained by Clauberg were one-fourth or one-fifth the value of the Allen unit. Rats and mice have been used as test animals, but

120. Corner, G. W.: Am. J. Physiol. **86**:74, 1928.
121. Corner, G. W., and Allen, W. M.: Am. J. Physiol. **88**:326, 1929.
122. Allen, W. M., and Corner, G. W.: Am. J. Physiol. **88**:340, 1929.
123. Allen, W. M.: Am. J. Physiol. **92**:174, 1930.
124. Clauberg, C.: Zentralbl. f. Gynäk. **54**:2757, 1930.
125. Wiesner, B. P.: Eugenics Rev. **22**:19, 1930.

according to Allen the changes in the endometrium are not sufficiently pronounced. Knaus [126] found that 0.5 Gm. of corpus luteum extract suppresses the uterine reaction of a pseudopregnant or pregnant rabbit weighing 2 Kg. to pituitary within forty-eight hours; hence his rabbit unit. Allen [78] criticized this method as technically too difficult and because the inhibition of pituitary may possibly be due to some substance other than the one which causes proliferation of the endometrium.

At present there exists some confusion as to the unit standardization, and therefore a statement concerning the number of units needs to be interpreted in terms of the method employed.

Many things have been inferred from experimentation with animals and applied by analogy to the human problem. Too frequently the gap between species has been bridged without proof or comment. Among the more important functions attributed to the corpus luteum are: the control of lactation, the stimulation of progestational proliferation and the preparation of the uterus for nidation of the fertilized ovum. The evidence relating to these separate functions may now be considered.

Lactation.—Much circumstantial evidence was accumulated over a period of years directing attention to the corpus luteum as a factor in stimulating the function of the mammary gland. At first it was reasoned that since the striking changes in the gland occurred only in association with pregnancy the changes must be dependent on some new structure appearing at that time. Therefore, the fetus, placenta and corpus luteum were each considered as logical possibilities. The corpus luteum gained much favor as the causative agent. Great variation according to species was noted. None of the workers, however, was able to produce convincing evidence of extensive proliferation resembling that seen in pregnancy or of active secretion of milk. Furthermore, women who had had their ovaries removed during pregnancy later lactated normally.

In recent years attention has been directed to the hypophysis, and gradually the corpus luteum has been excluded. In an excellent review of the subject Corner [127] summarized the status in 1930 as follows:

1. The administration to non-pregnant spayed rabbits of corpus luteum extract (progestin) did not cause proliferation of the mammary gland beyond the stage normally attained at puberty. The continuous presence of corpora lutea in the ovaries during a period equal to that of normal gestation (brought about by experimental means) was equally ineffective in altering the mammary gland.

2. Administration of extracts of whole sheep's hypophysis to spayed virgin rabbits caused proliferation of the mammary gland and simultaneous lactation.

126. Knaus, H.: Arch. f. exper. Path. u. Pharmakol. **151**:371, 1930.
127. Corner, G. W.: Am. J. Physiol. **95**:43, 1930.

producing in two weeks a condition scarcely distinguishable from that present at full term gestation. Previous preparation by action of the corpus luteum was found not to be necessary to this effect.

An excellent review of the subject by Turner [68] likewise excludes the corpus luteum as a causative agent.

The demonstration of prolactin from the anterior lobe of the pituitary gland by Riddle seems to have removed the corpus luteum from this field finally and conclusively.

Progestational or Progravid Proliferation.—Progestational proliferation, which is most conspicuous in rabbits, shows great variation according to species. The abundant and well known descriptions of this state in animals may be omitted here except for reference by way of comparison.

Different terms have been suggested: "premenstrual," "pregravid" and "progravid." Bartelmez [128] preferred Corner's "progravid, which is more euphonious and also expresses the generally recognized adaptative character of the condition."

In a survey of the literature Bartelmez [129] found that no real cytologic study had been made on any mammal. Furthermore, adequately preserved menstruating uteri are rare. For many reasons "one cannot, therefore, without great care, carry over the observations made on one species to another." Since there is normally variation in the length of the cycle there is doubt whether specimens taken outside of the period of actual menstruation can be truly dated except by reference to the preceding menstruation. Hence it is problematic how long before menstruation any given specimen was taken.

An excellent opportunity to study the relation of the corpus luteum to the uterus in the human being is provided by adequate preservation of the uterus and ovaries removed at operation when the data regarding the menstrual cycle are complete. A careful study with accurate description of seventeen such specimens was reported by Bartelmez,[129] who found variability in different regions of the same endometrium a constant feature during menstruation as well as at other stages of the cycle. He stated that "no theory of menstruation can ignore the variability observed in uteri removed at comparable stages during periods which appeared as expected," and that "when all reliable cases are taken into account, the only invariable menstrual changes in the uterine mucous membrane are vascular and involve rhexis . . ." He emphasized the need of much more detailed study of normal menstrual

128. Bartelmez, G. W.: Am. J. Obst. & Gynec. **21**:623, 1931.

129. Bartelmez, G. W.: Histological Studies on the Menstruating Mucous Membrane of the Human Uterus, Contrib. Embryol. no. 443, Carnegie Institution, Washington, 1933.

and intermenstrual extravasation before experimentally produced bleeding can be interpreted justly.

There is general agreement by many observers that removal of a corpus luteum during the latter half of the intermenstrual period is followed by uterine bleeding. The causal relation in these instances is not understood. Perhaps the bleeding is due to the withdrawal of theelin, which the corpus luteum has been shown to elaborate, or perhaps it is due to the loss of the progestin which is present in the corpus luteum. At present the manner of action is a matter of speculation.

Stimulated by the successful production of progravid endometrium in castrated animals by successive injections of theelin and progestin, several workers have tried similar experiments in women. Kaufmann [130] in 1932 announced the first successful production of a progravid condition of the endometrium in a castrated woman by means of theelin and progestin. One cannot say that he first produced menstruation, without entering into a controversy over the definition of menstruation, for uterine bleeding, as is well known, has been produced by the use of theelin alone. As just stated, Bartelmez maintained that the only invariable menstrual changes in the uterine mucosal membrane are vascular.

Later at a meeting of the Royal Society of Medicine, Kaufmann [131] described his experiments with women who had undergone bilateral ovariectomy but who retained their uteri intact. He reported that he was able to produce complete menstruation in castrated women by the administration of from 500,000 to 1,000,000 international units of estrogenic hormone followed by a small quantity of corpus luteum hormone. In the discussion that followed his presentation Dodds reported that he was able to confirm the experiments and had checked them by curettage. In the experiments of both Kaufmann and Dodds, while the material injected was specified in terms of international units of estrogenic hormone, it is to be noted that the material actually injected was di-hydro-estrin, a chemical compound derived in the laboratory from the estrogenic substance and not known to be elaborated by human tissues. The details concerning the corpus luteum preparations used and their dosage are too inadequate to permit critical interpretation here.

In addition to the treatment of castrated women, Kaufmann reported the successful treatment of patients with primary and secondary amenorrhea by derivatives of the estrogenic substance, alone as well as in conjunction with corpus luteum hormone. Hill, in discussing Kaufmann's paper, stated that he had used similar preparations in the same way and obtained satisfactory results in four cases of secondary amenor-

130. Kaufmann, C.: Zentralbl. f. Gynäk. **56**:2058, 1932.
131. Kaufmann, C.: Proc. Roy. Soc. Med. **27**:849, 1934.

rhea but had not obtained success in two cases of primary amenorrhea. Loeser [132] reported the production of menstruation in three women who had never menstruated or had any genital bleeding previously. He used 300,000 mouse units of follicular hormone followed by some corpus luteum hormone.

The data just presented are difficult to evaluate for the following reasons:

1. There is no unanimity of opinion as to what constitutes progravid changes, for secretion is not always present, glycogen may appear in the cells even before the corpus luteum is formed, and varying conditions are found in the same uterus at the same time.

2. There is no universally accepted definition of menstruation.

3. In the human being the lutein cell anatomically is distinctly of membrana granulosa origin while in some animals the theca cells participate to a greater or lesser degree.

4. It is difficult to explain why a lutein cell undergoing degeneration should take on an entirely new secretory activity and why the human lutein cell, known to have a high content of theelin, should secrete progestin, which is inhibited by theelin.

5. Since the same result is obtained by the use of chemically different compounds, the specificity of a hormone may be questioned.

6. No change in the ovary has been demonstrated following the administration of theelin, of its allied compounds or of progestin. It is therefore difficult to explain why administration of these compounds should be followed for several months by a normal cycle without further substitution.

7. Amenorrhea, both primary and secondary, is known to occur under widely varying circumstances, and the causes vary. In many instances there is no evidence that the corpus luteum is involved. Therefore, the same treatment can hardly be expected to produce the same results in all varieties.

Much has been inferred from experiments on animals, and although the analogies are often incomplete, a wide variety of human problems have been explained. Even tumors of the ovary and the breast have been classified because they seemed to show an activity attributable to progestin. Dysmenorrhea is alleged to be due to a lack of balanced activity of theelin and progestin.

Nevertheless, these experiments on human beings represent a definite advance in the elucidation of the problematic ovary-uterus relation. New problems for research are suggested. It is to be hoped that the

132. Loeser, A.: J. Obst. & Gynaec. Brit. Emp. **41**:86, 1934.

recent purification and crystallization of progestin added to the chemical advances in the preparation of the compounds related to theelin may facilitate the solution of these problems and furnish a sound basis for future therapy.

Nidation.—The importance of the function of the corpus luteum to the nidation of the fertilized ovum has been advocated since such a conception was announced by Prenant, von Born and Fraenkel. Nidation has frequently been used as a test for the activity of the corpus luteum. The relation between the corpus luteum and nidation in woman has been largely inferred from animal behavior. Rarely has species variation been taken seriously into account; nevertheless, it seems important to consider this. Since the corpus luteum is generally conceded to be the important factor in the ovary with reference to nidation, removal of the ovaries obviously eliminates this factor.

Drips [133] found that in the thirteen-lined ground squirrel, bilateral ovariectomy at any time in pregnancy caused interruption of gestation. The well known and often cited experiments of Fraenkel showed that in the rabbit removal of the ovaries or of corpora lutea up to the eighteenth day of pregnancy always interrupted gestation. Loeb [134] reported that in the guinea-pig gestation was interrupted only if the corpora lutea were removed before the end of the second day after coitus, even though implantation had not yet occurred. There are numerous instances in which the ovaries of the human being have been removed early in pregnancy without interrupting gestation. In one case, Pratt [135] removed the corpus luteum of pregnancy on the twenty-first day after the beginning of the last menstruation without any deleterious effect on the pregnancy. In this instance both ovaries were carefully inspected, and it is certain that no other corpus was present. The pregnancy terminated normally at the estimated time. At most, the corpus could not have been present longer than a few days during the implantation. These few citations illustrate well the wide species variation which has been too generally overlooked.

Aschoff [46] held as untenable the theory that the corpus luteum in some manner prepares the uterus for the fertilized ovum and further influences pregnancy. He maintained that the corpus does not determine the fate of the ovum, but that the ovum determines the fate of the corpus. Furthermore, he suggested that the corpus luteum possesses a local significance for the respective ovary: "Perhaps it concerns a particular regulative mechanism which, through the goodly storage of

133. Drips, D.: Am. J. Anat. **25**:117, 1919.
134. Loeb, L.: Am. J. Anat. **32**:305, 1923.
135. Pratt, J. P.: Endocrinology **11**:195, 1927.

lipoid and other substances, governs the chemical metabolic processes and the physical pressure-relationship in the ovary to the extent that through it the growth and ripening of the remaining follicles is influenced."

The doctrine of the "primacy of the ovum," championed by Meyer [43] and others since 1913, is related to the problem of nidation. The distinction between fertilized and unfertilized ova is not always clearly indicated.

The work of Zondek [136] on the hibernating bat has an important bearing on this subject. He wrote as follows:

> As a result of experimental investigations [Zondek, 1931] I have concluded as follows: "Ovum and hormone are coordinated in function, are not interdependent, but are both governed by the anterior pituitary lobe." That the gonadotropic anterior pituitary lobe hormone produces ripening of the follicle is now universally recognized. On the other hand, the question is again being discussed as to whether luteinization is caused by the anterior lobe hormone or whether a hormone is produced in the maturing ovum which stimulates the formation of the corpus luteum. This latter hypothesis [Clauberg, 1933; Schoeller, 1934] is not proved experimentally by its authors. According to this theory, a very important hormonic function would devolve upon the ripening ovum, viz., the development of the corpus luteum following rupture of the follicle and the production of progestin.

In experiments on the hibernating bat and on the rabbit Zondek found his results agreeing with those of Westmann, although derived in a different manner. They both showed that the formation of the corpus luteum is independent of the ovum. The following summary by Zondek includes several important facts:

> 1. Experiments on the ovary of the hibernating bat show that corpora lutea may be produced by means of prolan, although the ovum is pressed to one side or may be destroyed. From this it is already apparent that the formation of the corpus luteum cannot be dependent upon the ovum.
>
> 2. Direct proof may be given from the follicles of the sexually mature rabbit from which the ova are withdrawn by aspiration. After the injection of prolan such a follicle is converted into a corpus luteum. For the production of a corpus luteum it makes no difference whether an ovum is present in the follicle or not.
>
> 3. The formation of the corpus luteum can therefore not be dependent upon the ovum. The gonadotropic hormones of the anterior pituitary lobe effect ripening of the follicle and ovum, follicular rupture and development of the corpus luteum. The ovum plays no part in these hormonic events.
>
> 4. On the other hand, after it is fertilized, the ovum assumes a definite significance for the hormonic process. As a rule there follows the persistence of the corpus luteum which must be controlled by a reaction of the fertilized ovum on the anterior lobe of the hypophysis.
>
> 5. The great changes in the production of folliculin and prolan following on the fertilization of the ovum are neither conditional to nor consequent upon every

136. Zondek, B.: J. Physiol. **81**:472, 1934.

fertilization, since they do not occur as a general rule but only in certain animals. The mass excretion of oestrogenic hormone which has hitherto been determined only in association with a fertilized ovum can be found also in male animals of the equine species.

Many therapeutic ventures to prevent disturbances of nidation have been based on the assumption that the corpus luteum is essential to nidation. Until more factual evidence is adduced to show that a specific hormone is produced by the human corpus luteum which influences nidation, such therapy should be classed as empirical.

(To Be Concluded)

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Stanhope Bayne-Jones, professor of bacteriology, has been appointed dean of the Yale University School of Medicine in the place of Milton C. Winternitz, professor of pathology, who declined reappointment as dean.

The retirement is announced of Milton J. Rosenau, professor of preventive medicine and hygiene in Harvard University Medical School since 1909.

Bertram H. Buxton, professor of experimental pathology in Cornell University Medical College from 1904 to 1912, died at Devon, England, on Dec. 5, 1934, at the age of 82. He made important investigations on typhoidal infections, agglutination, absorption from the peritoneal cavity and plant physiology, and he was a pioneer as well as an expert in photomicrography.

Society News.—The Tenth International Congress of the History of Medicine will he held in Madrid, Spain, from Sept. 23 to 29, 1935.

Morton McCutcheon has been elected president, Esmond R. Long vice-president, and Herbert L. Ratcliffe secretary-treasurer, of the Philadelphia Pathological Society.

The American Association of Pathologists and Bacteriologists will meet at Cornell University Medical College, New York City, on April 18 and 19, 1935. One-half day will be devoted to the consideration of virus diseases in a joint meeting with the American Association of Immunologists. The headquarters will be Hotel Commodore.

Preparations are under way for the Second International Congress of Microbiology, to be held in London from July 27 to Aug. 1, 1936.

Karl F. Meyer has been elected president, Thomas M. Rivers vice-president, and L. L. Baldwin secretary-treasurer, of the Society of American Bacteriologists.

The Seventh International Congress on Industrial Accidents and Disease will be held in Brussels, Belgium, from July 22 to 27, 1935.

The American Association for the Advancement of Science will meet in Minneapolis from June 24 to 29, 1935, and in St. Louis from Dec. 30, 1935, to Jan. 4, 1936.

The American Society of Clinical Pathologists has arranged for a program of study of tumors in New York on June 3 to 6, preceding the annual meeting of the society in Atlantic City on June 7 to 9, 1935.

Ella Sachs Plotz Foundation.—In 1934, twenty-seven grants were made, fourteen of which went to workers outside the United States. In the eleven years of the existence of the foundation two hundred and twenty-seven grants have been made to investigators in twenty-four different countries. The maximum granted "will usually be less than $500." Applications for grants for 1935-1936 should be made before May 1 next to Dr. J. C. Aub, 695 Huntington Avenue, Boston.

National Research Council Grants.—Owing to the decreased resources at the disposal of the committee on grants-in-aid of the National Research Council, the committee will hold but one meeting this year, about the middle of May. Applications to be considered at this meeting must be in the hands of the secretary of the committee, Dr. C. J. West, 2101 Constitution Avenue, Washington, D. C., on or before April 1. Applications received after that date cannot be considered until the spring of 1936.

Abstracts from Current Literature

Microbiology and Parasitology

THE PHAGE-INACTIVATING AGENT OF BACTERIAL EXTRACTS. F. M. BURNET and G. A. C. GOUGH, J. Path. & Bact. **38**:285 and 301, 1934.

Bacterial extracts are in many cases capable of inhibiting the activity of phages lysing the parent strain. Extracts from resistant strains fail to inhibit the phages toward which the bacteria are resistant. The phage-inactivating power is destroyed by the addition of homologous antibacterial serum concurrently with the appearance of specific precipitation. The interaction between the active agent and the phage shows close resemblances to the phage-antiphage reaction in the time course of the reaction, in showing the "percentage law" of Elford and Andrewes and in giving evidence of the existence of "partially inactivated" phage.

The phage-inactivating agent of bacterial extracts can be purified and obtained free from protein and biuret-reacting substances. The product so obtained is a polysaccharide which can be converted into the usual hapten by treatment with alkali. During the course of degradation of the active polysaccharide by heat and alkali the power to inactivate different phages is lost in steplike fashion. The heat-stable and alkali-stable haptens show no phage-inactivating powers except for one phage, S 1. It is concluded that the specific somatic antigen of dysentery and salmonella bacilli is a complex labile polysaccharide which is responsible both for the serologic character and the phage-susceptibility of the bacteria.

FROM THE AUTHORS' SUMMARIES.

THE GENUS MONILIA. J. F. D. SHREWSBURY, J. Path. & Bact. **38**:313, 1934.

The fungi that Castellani has called "Monilia" are in no way related to the fungi belonging to the genus Monilia Gmelin or Persoon. Castellani's conception of a plurality of species in his genus is not supported by a study of certain of his species. The fungus that he has called Monilia Krusei, and which he lists as one of his type species, has no right to a place in his genus Monilia. Castellani's genus Monilia contains at least two distinct species which can be distinguished on cultural and biochemical grounds.

FROM THE AUTHOR'S CONCLUSIONS.

BACTERIAL FLUORESCENCE WITH ULTRA-VIOLET LIGHT. R. J. V. PULVERTAFT, J. Path. & Bact. **38**:355, 1934.

Under certain conditions ultraviolet light is of value in differentiating bacterial cultures. The cultures must be aerobic and grown on a medium incorporating the early breakdown products of the action of pancreatic extracts on meat. The period for which digestion must continue is in inverse ratio to the efficiency of the extract as tested on fibrin. Under these conditions organisms give either a green fluorescence, which is the usual finding, or a violet color. In certain cases of bacterial variation associated with a change in antigenicity and virulence, a change in fluorescence has been found, indicating a change in chemical composition.

FROM THE AUTHOR'S SUMMARY.

BACILLUS NECROPHORUS AND OXYGEN INTOLERANCE OF THE ORGANISM. W. I. B. BEVERIDGE, J. Path. & Bact. **38**:467, 1934.

The characters of the strains examined conform closely to those described by earlier observers. It has been possible to observe slight differences between some strains of this series, which reconcile different descriptions of these features in the

literature. Yet apart from serologic differences, slight differences in morphology and different cultural characters shown by strain I in broths and litmus milk, these strains constitute a uniform group. The differences brought out in the cross-agglutination tests find parallels in other species of bacteria. Strain I differs only in those features specially mentioned in the text; the smell of the cultures is characteristic, the biochemical properties are almost identical, and typical joint lesions (which Cesari considered to be pathognomonic for B. necrophorus) follow intravenous inoculations in rabbits.

FROM THE AUTHOR'S DISCUSSION.

THE BACTERICIDAL PROPERTIES OF ADRENAL EXTRACTS. J. GORDON and J. C. KNOX, J. Path. & Bact. **38**:493, 1934.

The results described in this paper indicate that after prolonged extraction of the bovine suprarenal gland with water at 65 C. without exclusion of air a substance is present in the dark-colored extract, which, when incorporated in a medium or allowed to act on a suspension of bacteria, has a marked inhibitory effect on the bacterial growth. This inhibition is most evident with Bacillus diphtheriae and Vibrio cholerae, but there is a wide range of other bacteria which show this inhibition in greater or less degree.

FROM THE AUTHORS' SUMMARY.

THE MONOCYTE-LYMPHOCYTE RATIO IN THE TUBERCULOUS GUINEA-PIG. KONRAD E. BIRKHAUG, Ann. Inst. Pasteur **51**:428, 1933.

The monocyte-lymphocyte ratio is closely related to the velocity of sedimentation, both being faithful indexes of activity in experimental tuberculosis. The monocyte-lymphocyte quotient and the velocity of sedimentation are markedly increased following a provocative dose of tuberculin in experimental tuberculosis. The relative avirulence of dissociated S variants of bovine tubercle bacilli is closely reflected in the benign monocyte-lymphocyte ratio and sedimentation reaction. The micro-sedimentation reaction permits a harmless and continuous procedure on laboratory animals and is a useful prognostic test in experimental tuberculosis. Intermittent bacillemia occurs in experimental tuberculosis and is best demonstrated by pooling the blood of a series of animals.

AUTHOR'S CONCLUSIONS.

ACTION OF SERUM OF DIFFERENT ANIMALS ON PNEUMOCOCCUS. PAUL BORDET, Ann. Inst. Pasteur **51**:503, 1933.

Recognized defensive reactions differs in various animals, owing to fundamental factors, and animals must hence be expected to respond differently to infections. Acidification is the most intense in the serum of animals that are most receptive to Pneumococcus. With serum of a rabbit, a receptive animal, large spherical bodies which could even pass for blood cells (color plate) appear, although they are not seen if the serum of the guinea-pig, horse or man is used. They are red by Laveran's staining method, and are rapidly soluble in bile, as are pneumococci. This was noted not only in cultures in rabbit serum, but in the blood and exudate of infected rabbits; on the other hand, these bodies were absent in cultures in rabbit antiserum. The production of the spherules could be demonstrated to depend on particular elements in the serum, thermolabile, adsorbed by aluminum hydroxide and precipitating with euglobulin. These principles are found in serum of other animals, and in immune serum, but activity is prevented by antagonistic factors, perhaps fundamentally involved in immunity.

INFLUENCE OF LOCAL INFLAMMATION ON TETANUS AND OTHER PROCESSES. M. GALEA and E. FALCHETTI, Ann. Inst. Pasteur **51**:673, 1933.

Inflamed lesions were invoked in guinea-pigs by avirulent tubercle bacilli or by grain infusions. Tetanus spores injected into these lesions spread more rapidly

than in normal animals. The spread was more rapid in the lesions caused by tubercle bacilli, but such areas were less favorable for spore germination. Lesions induced by the toxin of Clostridium oedematiens were rapid but less serious in animals with foci of inflammation than in controls, the lesions favoring elimination of the toxin. The virus of foot and mouth disease was retained in the skin, especially in lesions induced by B C G, longer than in the blood or the internal organs, but inflammatory processes did not affect the course of the infection. The authors see in these results a defense mechanism analogous to that occurring in vaccination with B C G.

FROM THE AUTHORS' CONCLUSIONS.

ACTION OF LIPOIDS IN EXPERIMENTAL TUBERCULOSIS. L. NÈGRE, Ann. Inst. Pasteur **51**:697, 1933.

Acetone extracts of lung, liver and kidney tissue activate the evolution of infection in tuberculous guinea-pigs. Other tissues yield a less active extract. There seemed to be little difference in the results secured by extracting organs of different animals. Although anatomic factors are probably responsible for the original localization of tubercle bacilli introduced subcutaneously into a guinea-pig, it is not unlikely that the presence of activating lipoid material in the lungs and kidneys accounts for the gravtiy of infection in these organs, a matter of clinical importance.

M. S. MARSHALL.

EXPERIMENTAL SYPHILIS IN THE GUINEA-PIG. J. VAN HAELST, Ann. Inst. Pasteur **51**:714, 1933.

Regional adenopathy was a constant symptom of genital syphilis in guinea-pigs. Following the initial reactions a predominant ganglion was noted in which spirochetes were found in numbers proportional to the age and extension of the exterior lesions. The division of spirochetes was analogous to that found in similar ganglions in man and in rabbits. Adenopathy developed in a manner parallel to that of the primary lesion, lasting on the average five weeks, but typical adenopathy was also induced without primary signs. Thus the guinea-pig showed progressive infection either with clear primary lesions or as inapparent infection following lymphatic drainage.

FROM THE AUTHOR'S CONCLUSIONS.

EXPERIMENTAL DIPHTHERITIC PARALYSIS. G. RAMON, ROBERT DEBRÉ and PIERRE UHRY, Ann. Inst. Pasteur **52**:5, 1934.

The paralyzing effect of mild doses of diphtheria toxin or of doses of partially detoxified toxin on animals has long been known. The typical reaction in a guinea-pig is an ascending paralysis which may eventually result in death from cardio-pulmonary complications. In rabbits paralysis was noted, but was similar to that noted with other bacterial toxins. The dog reacted in much the same way as the guinea-pig. The delayed paralytic reaction occurred simultaneously with antitoxin in the blood serum. Antitoxin was effective only when administered well in advance of the fixation of toxin by nerve tissues. Methenamine, sodium salicylate, thyroxine and methylthionine chloride U. S. P. (methylene blue) failed to prevent paralysis. The symptoms were considered to be due to typical toxin. Results depended solely on the dosage. The fixation of toxin by nerve tissues seemed to occur within less than twenty-four hours after injection, although paralysis did not ordinarily appear before the third week.

M. S. MARSHALL.

EXPERIMENTAL STUDY OF HEAT THERAPY. C. LEVADITI, H. DE ROTHSCHILD and Others, Ann. Inst. Pasteur **52**:23, 1934.

Spirilla, spirochetes, protozoa and bacteria were used as infecting agents, particularly the spirochete of syphilis, in rabbits and mice. Short wave radiation either cured or retarded infections of rabbits with Toxoplasma cuniculi and

lymphogranulomatous encephalitis of mice, but in acute and promptly fatal infections, such as spirillosis of chickens, trypanosomiasis (Tr. evansi) or ultravirus infections (herpes, rabies) results were nil. In experimental syphilis it was noted that the original lesion might heal and spirochetes disappear from it, the organisms might change morphologically, foci might be sterilized, and serologic reactions might become negative, but these results were by no means constant. The reaction is thus individual, and the authors express a belief that the action is one not of direct sterilization but of stimulation of the defense factors of the hosts, some of which respond. The reaction is similar in principle to that of some forms of chemotherapy. In syphilis, the results were not considered equal to those from chemotherapeutic measures. Only the results from general radiation were determined, not those from radiation as applied to specific foci.

FROM THE AUTHORS' "GENERAL CONSIDERATIONS."

INTRAPERITONEAL INJECTION OF RABIES VIRUS. A. C. MARIE, Ann. Inst. Pasteur **52**:141, 1934.

The injection of Chinese ink subcutaneously or into the blood stream of guinea-pigs had been previously shown by the author sometimes to facilitate and sometimes to prevent rabies when the virus was injected intramuscularly. Intraperitoneal injection of Chinese ink, however, reduced the resistance of the serous surfaces of the peritoneal cavity by blocking the reticulo-endothelial system to such a point that infection with a fixed virus took place. Van Deinse's mixture of disodium phosphate and calcium chloride was also successful for blocking. The results were clearcut, and the controls confirmed observations that intraperitoneal injections of rabies virus rarely result in infection.

M. S. MARSHALL.

CHEMICAL STUDIES OF THE TUBERCLE BACILLUS: I. M. MACHEBOEUF, G. LÉVY, N. FETHKÉ and Others, Ann. Inst. Pasteur **52**:277, 1934.

This first report of a planned intensive study deals with extraction and fractioning of lipoid substances from a kilogram of moist, heat-killed organisms. The method of preparation and some properties are noted for five fractions, A to E, some of which were again subdivided: A, solvent in acetone, 11 Gm., a fatty phosphorus-free material, with pigments and no haptens; B, solvent in cold alcohol, 7.1 Gm., a fatty, phosphorus-containing substance, with strong haptens; C, solvent in hot alcohol, 5.4 Gm., a hard waxy mass, weak in phosphorus and decidedly acid-fast; D, solvent in ether, 5.2 Gm., a transparent glassy substance, strong in phosphorus, and yielding reducing sugars and other substances on hydrolysis; E, solvent in chloroform, 1.8 Gm., also a transparent glassy material, strong in phosphorus, and yielding reducing sugars and other substances on hydrolysis.

M. S. MARSHALL.

CHRONAXIA OF EXPERIMENTAL POLIOMYELITIS. A. AND B. CHAUCHARD, B. ERBER and P. MOLLARET, Ann. Inst. Pasteur **52**:444, 1934.

The latest model of the Lapicque apparatus, with a capacity of 90 volts and possible reduction to 1/200 of the voltage, was used on monkeys. The apparatus appeared to measure adequately the neuromuscular excitability of the normal monkey. In order to prevent possible paralysis arising from injury to the brain, the authors resorted to intraperitoneal injections of the virus. There was a rigorous parallelism between the values of chronaxia and the evolution of paralysis. Since measurements indicated reaction before paralysis could be noted, during the period of incubation, a cerebral disturbance was indicated before infection was manifested, an infection which might become abortive or inapparent. In other words, "it demonstrates beyond doubt the constancy of a precocious encephalitic dissemination of the poliomyelitis virus until now unsuspected."

M. S. MARSHALL.

MOOSER BODIES AND RICKETTSIAE IN MEXICAN TYPHUS. RUDOLF WEIGL and A. HERTZIG, Arch. Inst. Pasteur de Tunis **22**:321, 1933.

These bodies, found in the endothelial cells of the tunica vaginalis of the guinea-pig or the rat infected with Mexican typhus, were considered to be identical with rickettsiae, which are thought to differ from those of European typhus. The authors propose tribute to Mooser's contributions by replacing the term "Mooser's bodies" with "Rickettsia Mooseri." In a subsequent note (p. 325) Nicolle goes further in proposing "Mooser's cells" as a designation for the typical cells with inclusions, analogous to lepra cells of lepromas. M. S. MARSHALL.

PASSAGE OF TYPHUS VIRUS VIA THE DIGESTIVE TRACT. CHARLES NICOLLE, J. LAIGRET and P. GIROUD, Arch. Inst. Pasteur de Tunis **22**:326, 1933.

Strains of rodent virus infected rats quite regularly by way of the digestive tract, although other viruses were less frequently thus transmitted. The virus of Old World typhus, for example, is regarded as having adapted itself to man. When infected fleas were ingested instead of brain tissue, which was used earlier, rats were infected. Fleas of the species Xenopsylla cheopis were infected with the rodent virus, and the bites of the infected fleas produced infection of one of two monkeys. It is believed, however, that the usual method of transmission in rats is by way of the digestive tract, either through cannibalism or by ingestion of the ectoparasites. Attention is again drawn to the importance of silent or inapparent infection, manifested in most of the test animals. Without recognition of this state, most of the animals would have been considered uninfected.

CULTIVATION OF TYPHUS VIRUS. L. A. ZILBER and E. M. DOSSER, Arch. Inst. Pasteur de Tunis **22**:486, 1933.

It is indicated that at the Metchnikoff Institute in Moscow the virus was cultivated in ordinary infusion peptone broth in the presence of Torula kéfir and to a lesser extent with several bacteria. A sixteenth generation is mentioned. Success was measured in terms of animal inoculations, with histologic confirmation and demonstration of rickettsia bodies, louse infection and cross-immunity. Rickettsia bodies were not found in the cultures. M. S. MARSHALL.

PRESERVATION OF THE TYPHUS VIRUS AT LOW TEMPERATURE. JEAN LAIGRET and ROGER DURAND, Arch. Inst. Pasteur de Tunis **22**:499, 1933.

The virus of Rocky Mountain spotted fever survived in blood or brain tissue at temperatures of from —12 to — 15 C. for thirty-five days, and other viruses survived twenty days. Glycerin, heretofore considered destructive, was resisted perfectly at this temperature, and because of its bactericidal properties is recommended. M. S. MARSHALL.

TYPHUS VIRUS IN RAT URINE. CHARLES NICOLLE, PAUL GIROUD and HÉLÈNE SPARROW, Arch. Inst. Pasteur de Tunis **23**:1, 1934.

Using three rodent strains of typhus virus, two of them Mexican, Nicolle, Giroud and Sparrow inoculated ninety-three rats. Urine from the infected rats was used to inoculate guinea-pigs by various routes. The strain most frequently tested (seventy-four rats), a Mexican type (Zinsser), infected guinea-pigs in two instances, once on exposure of the mucous membrane of the eye for four minutes and once on injection of 0.3 cc. into the inferior palpebral conjunctiva. The concentration of the virus in rat urine appeared to be small, but the possibility that such transmission occurs among wild rats or that laboratory infections may arise in this manner cannot be overlooked. M. S. MARSHALL.

AN ORGANISM FROM TUNIS RESEMBLING BACILLUS TULARENSIS. CHARLES ANDERSON, Arch. Inst. Pasteur de Tunis **23**:53, 1934.

An organism, apparently identical with B. tularensis, with which it was compared, and even showing a serologic affinity with Brucella melitensis, was isolated from a wild rabbit found dead in Tunis.

GRANULAR BLENNORRHEA: II. UGO LUMBROSO, Arch. Inst. Pasteur de Tunis **23**: 60, 1934.

This report concludes a careful study of an eye infection, not due to the gonococcus, designated on clinical and histologic grounds as granular blennorrhea. It is considered to arise in the new-born, by way of the genital passages, manifesting itself after an incubation of from two to forty days, and continuing as described in an earlier paper (*Arch. Inst. Pasteur de Tunis* **22**:513, 1933). There are no corneal lesions. Silver nitrate does not serve for prophylaxis. Although pathogenic organisms complicate the disease, neither the bacteria nor the fungi encountered were considered specific. The disease is contagious, the virus apparently passing fine Berkefeld filters. It is not known whether immunity occurs, either specific or against trachoma. Epithelial cell inclusions occur just as in trachoma and in other diseases of the conjunctiva. Histologically, acute lesions show regressive and necrotic changes involving the epithelium, the tunica propria and the conjunctiva, and in subchronic lesions there appear granulations showing almost no tendency toward regression, characterized by lymphoid elements and histiocytes. The virus is not widely destructive or particularly invasive, but its survival is so great that the prognosis is, in this sense, grave.

FROM AUTHOR'S GENERAL CONCLUSIONS.

CULTIVATION OF THE VIRUS OF FOOT AND MOUTH DISEASE IN TISSUE CULTURES. FRIEDRICH HECKE, Zentralbl. f. Bakt. (Abt. 1) **126**:93, 1932.

Hecke succeeded in cultivating the virus of foot and mouth disease in tissue cultures. The virus increased but slowly when the tissue culture contained very little tissue, but became larger in amount in the presence of larger amounts of tissue. The virus was present in higher concentration in the tissue than in the surrounding fluid, indicating that the increase in virus occurs in the tissue. The p_H of the cultures dropped in from 5 to 6 days from 7.5 to 6.9 or less during the time in which the virus was increasing, the highest concentration of virus being obtained at p_H 6.9. The concentration of virus decreased at a lower p_H, and below p_H 6.3 virus could not be demonstrated. Death of the tissue caused a strong basic reaction and destruction of the virus.

PAUL R. CANNON.

BACTERIOLOGIC INVESTIGATION IN 400 NECROPSIES. J. PUTNOKY, Zentralbl. f. Bakt. (Abt. 1) **126**:248, 1932.

Putnoky made a systematic bacteriologic examination of tissues in 400 necropsies, using cultural and staining methods. Bacteria were demonstrable in different organs in 68 per cent of the cases by the combined method and in 55 per cent by cultural methods alone. The cultures were taken from the cardiac blood, splenic pulp, gallbladder and elsewhere. He concludes that bacteriologic examination is of value in determining the source of entrance of bacterial infection, and that it is reliable up to fifty-five hours after death and is more reliable in the cooler months of the year. He found hematogenous infections of the gallbladder a common event, and that peritoneal inflammation or abdominal operations increase the chances of blood stream infection.

PAUL R. CANNON.

CULTIVATION OF SWINE PEST VIRUS IN TISSUE CULTURES. FRIEDRICH HECKE, Zentralbl. f. Bakt. (Abt. 1) **126**:517, 1932.

Hecke succeeded in cultivating the virus of swine pest in tissue cultures and describes his methods in detail. Drop cultures, made with the choroid plexus of

young swine in swine plasma and extract of swine spleen, were infectious through fifteen passages. Flask cultures with lymph nodes were infectious through twenty culture generations. The flask cultures were maintained at 37 C. for one hundred and five days, and the material was thereby diluted 10^{30}. Cultivation of the virus by means of kidney tissue did not succeed. The virus was demonstrable in control cultures at 37 C. without tissue up to eighteen days. Transfer to other similar cultures resulted in loss of the virus in the second generation.

PAUL R. CANNON.

VITAL PROCESSES OF SPIROCHAETA PALLIDA IN VITRO. E. J. ROUKAVITSCHNIKOFF, Zentralbl. f. Bakt. (Abt. 1) **126**:530, 1932.

The author observed the transformation of two strains of spirochetes isolated from syphilitic lesions into masses of almost formless substance in which swollen and globular forms exhibiting a slight motility were demonstrable. Subculture gave typical forms in some instances and atypical forms in others. He concludes that these spirochetes possess the abilty in vitro to undergo marked involutional changes, but that in the presence of endogenous stimulation of an unknown nature they may resume their former vital activity. Although he gives no evidence, he apparently assumes that the micro-organisms he observes are actually varying forms of Spirochaeta pallida.

PAUL R. CANNON.

DESTRUCTION OF VACCINIA VIRUS BY LIGHT ENERGY AND DYES. K. HERZBERG, Ztschr. f. Immunitätsforsch. u. exper. Therap. **80**:507, 1933.

The virucidal effect of various dyes, notably methylthionine chloride U. S. P. (methylene blue), was greatly enhanced by combination with light, particularly with ultraviolet rays. Different experimental conditions and results are described and evaluated.

I. DAVIDSOHN.

UNDULANT FEVER AMONG STUDENTS AND LABORATORY WORKERS. O. HERRMANN, A. MIRSABEKJAN and R. MEGRABJAN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:500, 1934.

Herrmann and his associates claim that this is the first report of the occurrence of undulant fever in man in Armenia. The infection of cattle, horses, sheep and donkeys had been reported previously. Of twelve persons who were engaged in the laboratory (7 physicians, 3 technicians and 2 assistants) four contracted the disease (three physicians and one technician). In three others who did not feel ill there was a rise of specific agglutinins in the blood serum, and one of the three proved to be a carrier. A study of a large group (673) of students of the veterinary and medical schools revealed in 6.3 per cent the presence in the blood of agglutinins for Brucella abortus in a titer of at least 1:100; in 13.5 per cent agglutinins were present in borderline titers and in 80.2 per cent no agglutinins were shown. The incidence of positive agglutination reactions was in direct proportion to the frequency and intimacy of the contact of the students with infected cattle. Among those with a positive agglutination reaction only twenty-six had symptoms at the time of the study or in the past, while sixteen never felt ill, although one of them had a titer of 1:5,000 and the blood of another infected a guinea-pig. Because of the absence of fever in many cases and the lack of undulation in others, Herrmann and his associates suggest that the term "undulant fever" be abandoned.

I. DAVIDSOHN.

STRUCTURAL CHANGES IN BRUCELLA SUIS INFECTION IN SWINE. M. J. CHRISTIANSEN and A. THOMSEN, Acta path. et microbiol. Scandinav., supp. 18, p. 64, 1934.

The principal structural changes in the organs examined were indurative metritis, orchitis, epididymitis, seminal vesiculitis, with suppurative foci in dilated

glandular tubules, which were filled with necrotic cells, and focal, partially calcified necrosis; also indurative partially necrotic fetal placentitis.

Pathologic Anatomy

ATHEROMA AND CORONARY THROMBOSIS IN A YOUNG DIABETIC. E. R. CULLINAN and G. GRAHAM, J. Path. & Bact. **38**:167, 1934.

A search of the literature has not shown any account of a case in which there was such widespread arterial damage and death from coronary thrombosis at such an early age. The cause of the arterial damage cannot be assigned. The incidence of arterial degeneration and the knowledge that the blood cholesterol was raised were two of the factors which led to the recent introduction of a diet richer in carbohydrate and poorer in fat for the treatment of diabetes. Unfortunately in our case the cholesterol content of the blood was not estimated. Also the relationship between exogenous cholesterol and atheroma is far from clear. It is possible by cholesterol feeding to produce atheroma-like lesions in rabbits and sometimes in rats and mice, but not in cats or dogs, and it is almost certain that Carnivora have means of dealing with cholesterol which Herbivora lack. It may even be that atherosclerosis in the human subject is associated with an upset, not of the exogenous, but of the endogenous, metabolism of cholesterol. This case illustrates, in a man who had had relatively mild diabetes, widespread arterial atheroma with coronary thrombosis and death at the exceptionally early age of 27.

THE RETICULUM IN TUBERCLES IN THE HUMAN LIVER. T. D. DAY, J. Path. & Bact. **38**:175, 1934.

The reticulum associated with miliary tubercles in the human liver is found to be either a new formation associated with healing or the remains of that which supported preexistent liver cells. The presence of reticulum similar in arrangement to that of the normal liver in situations from which all trace of liver cells have disappeared is considered to indicate a previous necrosis. Such reticulum was present in 90 per cent of all the lesions examined. It was best seen in caseous material, and it intimately surrounded the majority of giant cells. It is concluded that necrosis must in many cases have preceded giant cell formation. Evidence is put forward to show that the substance of giant cells differs from caseous material.

FROM THE AUTHOR'S SUMMARY.

THE HEIGHT AND WEIGHT OF TUBERCULOUS CHILDREN. VELMA SPAULDING, Tubercle **15**:22, 1933.

Comparison of the author's material with that of Swanson, Emerson-Manny and Bardeen (used for controls) and with other data from the literature indicates that tuberculous children resemble average children closely in trends of average height, weight and index for age, index for height, and weight for height, and slightly in trends of variability, increment and rate of growth of height and weight per year, and that the tuberculous children appear taller and lighter (at most equal in weight) for age and slightly more slender relatively (as measured by the index of body build) than the comparative series. The groups of tuberculous children seem to show growth in height and weight in most respects much lige average children selected from the literature. They show no tendency to a retardation of growth in height, and while they appear slightly more slender than average children, they do not, in view of the inadequacy of the data, present conclusive evidence of a habitus phthisicus. FROM THE AUTHOR'S CONCLUSIONS (H. J. CORPER).

DESTRUCTION OF RED CELLS. A. POZZAN, Pathologica **25**:331, 1933.

Examination of the lymph glands of 120 newly born children showed that the reticulo-endothelial cells of the glands take an active part in the destruction of red blood cells.

E. VON HAAM.

LIPOPHAGIC GRANULOMA. G. H. BARTSCH, Arch. f. klin. Chir. **178**:62, 1933.

Granulomatous growths may originate about areas of focal necrosis in the subcutaneous fat, which has become saponified and acts as a foreign body. These growths occur usually after trauma and are not infrequent in the breast, and in elderly women they may be mistaken for carcinoma. There may even be an associated retraction of the nipple. Extramammary growths may be mistaken for sarcoma.

JACOB KLEIN.

INVOLVEMENT OF THE FIFTH AND TENTH CRANIAL NERVES IN FUSOSPIROCHETAL INFECTIONS AND IN CARCINOMA. E. v. BALOGH, Beitr. z. path. Anat. u. z. allg. Path. **92**:25, 1933.

Balogh attaches to the fusiform bacilli the predominating rôle in fusospirochetal infections. In a case of noma of the cheek seen a number of years ago, exacerbation of the process after a stage of progress toward healing led to examination of the gasserian ganglions. The ganglion of the affected side contained many fusiform bacilli. In the succeeding years the gasserian ganglion has been examined in noma and carcinoma of the face, and the tenth nerve and its ganglion in fusospirochetal gangrene and in carcinoma of the lung. Histologic evidence thus obtained indicates that propagation of the pathologic process may occur along the blood or lymph vessels of both nerve paths. Involvement of the nerve or its ganglion may lead to trophic changes that are an important factor in the gangrenous fusospirochetal lesion. Transport along the tenth nerve may account for metastatic inflammatory lesions of the meninges or brain in gangrene of the lung and for secondary intracranial tumors in carcinoma of the lung.

O. T. SCHULTZ.

HISTOPATHOLOGY OF GASTRO-INTESTINAL LESIONS OF PYOCYANIC INFECTION. S. BÉZI, Beitr. z. path. Anat. u. z. allg. Path. **92**:41, 1933.

The gastro-intestinal lesions in two fatal cases of B. pyocyaneus infection are described. In one case the portal of entry was the tonsil; in the other, the pharynx. In the stomach and intestine the infection may lead to the formation of isolated small ulcers or to larger and more diffuse necrotic lesions of the mucosa. Infection of the gastro-intestinal tract may occur by way of the blood stream, by the swallowing of the organisms or by their excretion into the gallbladder.

O. T. SCHULTZ.

GASTRO-INTESTINAL LYMPHOGRANULOMATOSIS. A. DUDITS, Beitr. z. path. Anat. u. z. allg. Path. **92**:59, 1933.

Five cases of lymphogranulomatosis with predominant involvement of the gastro-intestinal tract are reported. In three the stomach was the chief seat of the disease, and in two, the intestine. The mucosa revealed only a nonspecific granulomatous reaction, the specific lesion being in the submucosa or deeper tissue. In gastro-intestinal lymphogranulomatosis the process reaches the tract by direct continuity from the involved mesentery, by retrograde lymphatic transport from the mesenteric nodes or rarely by primary involvement of the lymphoid tissue of the tract.

O. T. SCHULTZ.

CIRRHOSIS OF THE LIVER IN THYROTOXICOSIS. G. HABÁN, Beitr. z. path. Anat. u. z. allg. Path. **92**:88, 1933.

In ten of twenty-six fatal cases of thyrotoxicosis the liver revealed varying degrees of interlobular fibrosis; the process was most marked in the subcapsular tissue. Habán believes that this form of fibrosis is to be distinguished histologically from other forms of cirrhosis and proposes for it the name "cirrhosis basedowiana." Fatty change, focal necrosis and interlobular round cell infiltration are evidence of toxic action, to which the cirrhosis is also due.

O. T. SCHULTZ.

FORMATION AND NATURE OF ASBESTOSIS BODIES. P. J. BERGER, Virchows Arch. f. path. Anat. **290**:280, 1933.

Recent literature describes as characteristic and histologically diagnostic of asbestosis of the lung the presence in the tissue of golden yellow bodies whose relation to asbestos has not been clearly determined. Berger presents an exhaustive morphologic and physicochemical study of these bodies. The bodies vary greatly in size and shape. They are thicker and longer than asbestos particles. They usually have clubbed or rounded ends. Running through the center of the body there is sometimes seen a row of globules like a string of pearls. The inspired asbestos dust lodges in the tissues of the lung in the form of needles of variable length. The magnesium is dissolved out by the action of the tissue fluids, leaving behind the silicate framework of the needle. At this stage the needles retain their original form and are still doubly refractile. From the silicate remains of the needle a substance, presumably colloid silicic acid, is adsorbed by the protein of the fluid in which the needle lies. This leads to the precipitation of a gel about the remnants of the needle. The silicate portion of the needle is dispersed in the gel. In this way the entire needle may be resorbed except for some granular iron oxide, which gives the asbestosis body its color. The formation of the asbestosis bodies should lead to the removal of asbestos from the tissues. Berger believes that in a percentage of workers in asbestos complete removal does occur and no harm results. In others, because of the complexity of the physicochemical system involved in the removal, complete resorption of the asbestos does not occur and a chronic inflammatory reaction is set up. He holds that asbestosis is a special form of silicosis, and that it is an occupational disease for which the victim should receive compensation.

O. T. SCHULTZ.

A CASE OF TRUE HERMAPHRODITISM. K. STOJALOWSKI and J. DEBSKI, Virchows Arch. f. path. Anat. **290**:358, 1933.

The authors tabulate nine previously reported examples of true hermaphroditism. In all but one the gonad was a combined ovary and testis. In their patient, who was operated on because of a diagnosis of incarcerated right inguinal hernia, there was a hypoplastic uterus and a right ovariotestis, with graaffian follicles and corpora haemorrhagica, and seminal tubules with stages of spermatogenesis but no spermatozoa. In the rudimentary scrotum was a small left testis from which by puncture living spermatozoa were obtained. This patient menstruated regularly from a hypospadiac urethra. The breasts and escutcheon were of the female type, but the body build was male. The sexual feeling was male. Nocturnal emissions had occurred from time to time, and the subject had had intercourse with women, with orgasm and ejaculation.

O. T. SCHULTZ.

HISTOLOGIC CHANGES INDUCED BY OVERDOSAGE OF VITAMIN A. G. DOMAGK and P. VON DOBENECK, Virchows Arch. f. path. Anat. **290**:385, 1933.

In rats that received daily by mouth 100,000 units of vitamin A concentrate, the Kupffer cells of the liver, the pulp cells of the spleen and the capillary endothelium of the kidney and lung contained fat. This change is ascribed to increased fat resorption brought about by the vitamin. The squamous epithelium of the proventriculus became hyperplastic, its cells appeared immature, and hornification disappeared. The changes described occurred irrespective of the oil used as the solvent for the vitamin concentrate. The oils alone, sesame, peanut and paraffin oils, had no such effects.

O. T. SCHULTZ.

CHANGES IN CARTILAGE IN GENERALIZED DISEASES OF BONE. G. SCHMORL, Virchows Arch. f. path. Anat. **290**:396, 1933.

In acromegaly Erdheim has described proliferative changes in joint and other cartilages which he ascribes to the action of the same endocrine factors that cause

the osseous changes. In the course of his investigations on the pathology of bone Schmorl had brought together, shortly before his death, observations on alterations in cartilage in osteomalacia, Paget's osteitis deformans and von Recklinghausen's osteitis fibrosa. The changes noted consisted in proliferation of the cartilage cells of joint and rib cartilage, together with a return of endochondral bone formation. Similar progressive changes, associated with regressive alterations, were noted in the intervertebral disks. Schmorl ascribes the reactions noted to mechanical factors.

O. T. SCHULTZ.

MYCOTIC ANEURYSM OF THE PULMONARY ARTERY. W. STEINBERG, Virchows Arch. f. path. Anat. **290**:433, 1933.

A woman, aged 36 years, became ill nine months before her death with fever of unknown origin. From that time on she was troubled with dyspnea and cardiac symptoms. Nephritis was diagnosed shortly before death. Necropsy revealed a purulent pericarditis. In the root of the pulmonary artery, just above the left valve segment, an aneurysm, with dimensions of 3 by 2.5 cm., protruded into the pericardial cavity. The aneurysm was filled with polypoid thrombotic material, in which and in the pericardial exudate streptococci were found. Near the mouth of the aneurysm was a small area of healing endarteritis. A detailed microscopic study of the lesion is presented. The outer portion of the wall of the artery in the region of the aneurysm had been destroyed by a small abscess, which is held to have been the cause of the aneurysm. The infection is believed to have had its origin in the illness of nine months before death. Steinberg could find records of only six cases of mycotic aneurysm of the pulmonary artery in the literature, none with a full description of the histologic picture.

O. T. SCHULTZ.

ARTERIOSCLEROSIS OF THE LIVER. C. PAPP, Virchows Arch. f. path. Anat. **290**:551, 1933.

The hepatic alterations in two cases of generalized arteriosclerosis are described in detail. Ten additional cases are summarized in tabular form. Macroscopically the arteriosclerotic liver is hard, somewhat decreased in size and weight, and sometimes slightly altered in form. The microscopic picture is considered characteristic. The entire arterial system of the liver may be involved in the arteriosclerotic process, or the latter may be limited to the medium-sized and small vessels. The decreased arterial supply leads to degeneration, atrophy and disappearance of liver cells, and to their replacement by reticulum. The latter never forms collagen. The collagenous tissue of the liver is unchanged. The scanty literature dealing with the subject is reviewed. Concerning the clinical manifestations of hepatic arteriosclerosis nothing is known.

O. T. SCHULTZ.

CYTOLOGY OF THE RENAL CONVOLUTED TUBULE. A. TERBRÜGGEN, Virchows Arch. f. path. Anat. **290**:574, 1933.

Terbrüggen devotes seventy-four pages and thirty-eight illustrations to a cytologic study of the epithelium of the proximal, or main, convoluted tubule of the kidney of the mouse and the rat under normal conditions, in states of hunger and thirst, and after water or urea diuresis. Especial attention is paid to the Golgi apparatus, which he claims has never been previously demonstrated in the human kidney. He describes its characters in the human fetal and adult kidney under varying conditions of health and disease. In the brush border of the cell temporary openings appear from time to time through which excreted material is delivered. The cytoplasmic rods are never granular in properly fixed preparations; they are held to be plastosomes. Granuloid is not a definite morphologic structure. It consists of uriniferous excretory material; in part it may be the result of swelling of cytoplasmic colloid. The Golgi apparatus is situated near the nucleus. Under varying states of activity it may enlarge and occupy the basal

or distal part of the cell. It is a concentrating organella, and not an excretory or secretory apparatus. In animals vitally stained with trypan blue, the dye is taken up by the tubules from the lumen and in time passes to the base of the cell. The region occupied by the Golgi apparatus may be deeply stained, but the apparatus itself is unstained. The hyaline droplets of the cytoplasm are foreign protein destined for excretion. The material is formed first in the meshes of the Golgi apparatus, which concentrates the material. In the lumen the droplets may take part in the formation of casts. The presence of multiple small droplets in the cytoplasm is the result of a degenerative process which begins in the most proximal portion of the main convoluted tubule. In this process the rods and Golgi apparatus degenerate, disrupt and may disappear. O. T. SCHULTZ.

PATHOLOGIC HISTOLOGY OF AINHUM. YÜEN DJIN DSCHANG, Virchows Arch. f. path. Anat. **290**:648, 1933.

A brief survey of the conflicting views on the etiology and nature of ainhum is followed by a summary of the author's findings in the histologic study of toes in thirteen cases of the disease. The chief changes noted were diffuse fibrosis of all the tissues of the toe and hypertrophy of the overlying epidermis. These alterations were not histologically specific, but they were sufficiently characteristic to distinguish the process from leprosy and other diseases with which ainhum has been confused. No micro-organisms, fungi or parasites were seen in the tissue. The etiology remains unknown. The author surmises that the disease may be due to functional factors, either circulatory or nervous, that do not document themselves by characteristic tissue reactions. O. T. SCHULTZ.

NECROBIOSIS OF THE HYPOPHYSIS IN INCREASED INTRACRANIAL PRESSURE. E. J. KRAUS, Virchows Arch. f. path. Anat. **290**:658, 1933.

In earlier and previously abstracted work Kraus laid down the rule that increased intracranial pressure of sufficient degree and duration, due to tumor or hydrocephalus, leads to hyperplasia of the adenohypophysis if the hypophyseal stalk has not been injured by the pressure. Small areas of necrosis in the anterior lobe are not rare. In the systematic microscopic examination of the hypophysis in 150 cases of increased intracranial pressure, the results of which he records, minute circumscribed areas of necrobiosis in the posterior lobe and especially in the stalk at its attachment to the neurohypophysis were not infrequently seen. Similar changes have been described by Weisz in the midbrain. These changes in the midbrain and hypophysis Kraus ascribes to the mechanical action of the increased pressure. There were no clinical manifestations that could be correlated with the necrobiotic process. O. T. SCHULTZ.

ANOMALIES OF THE CORONARY ARTERIES. E. BORN, Virchows Arch. f. path. Anat. **290**:688, 1933.

Born describes five examples of anomaly of the coronary arterial system of the heart. In two, the right and left arteries had a common ostium, the first portion of the left taking an unusually deep course through the muscle before becoming superficial. In the third case, in which death was sudden, the aortic valve had only two segments, and the circumflex artery took an unusual course about the root of the aorta; compression of this vessel was believed to have been the cause of death. In another heart with a two-segment aortic valve, the sinus of Valsalva from which both coronary arteries arose was aneurysmal. In the fifth specimen the origin of the left artery was unusually high above the aortic ring. Born believes that sinus aneurysm or unusually high origin of a coronary artery may seriously interfere with the coronary circulation and lead to infarction of the myocardium. O. T. SCHULTZ.

RELATION OF TORTUOSITY TO ARTERIOSCLEROSIS OF THE SPLENIC ARTERY. W. SPRINGORUM, Virchows Arch. f. path. Anat. **290**:733, 1933.

To the generally accepted view that tortuosity of arteries is the result of arteriosclerosis objection has been made by M. B. Schmidt and others, who claim that the tortuosity is due to inherent constitutional structural peculiarities of the vessel wall. To investigate the possible relation between tortuosity and arteriosclerosis Springorum made a study of the splenic artery in fifty subjects, all but four of whom were over 40 years of age. This artery was selected because of the frequency with which tortuosity of its course is observed. Gelatin was injected into the vessels in situ, and they were fixed in formaldehyde solution. To express the degree of tortuosity there was devised the formula $I=\frac{L}{100\,A}$, in which *I* is the index, *A* the length of the artery from its origin to its final branching before it enters the spleen, and *L* the direct or airline measurement between these two points. Vessels with an index of 300 or over were markedly tortuous, taking either a spiral course or one with variable bends in the same plane. Tortuosity is due to an increase in length. The latter may be actual or relative. Actual increase in length may be from stretching of the vessel as the result of loss of elasticity or from growth of the artery. In the former case the vessel is thin-walled; in the latter, thickened. The degree of tortuosity bears no relation to age and hence is not due to arteriosclerosis of the diffuse type, since the latter increases with age. Nodose arteriosclerosis is observed in arteries with an irregularly bent course, the areas of thickening being situated on the convex side of the bends. Nodose arteriosclerosis is the result and not the cause of tortuosity. Springorum thinks that elongation of the artery by stretching is due to the action of toxic factors on the wall of the vessel. Increase in length by growth of the artery is usually associated with enlargement of the spleen, and is held to be the result of increased functional demands on the part of the enlarged spleen. Relative increase in the length of the splenic artery occurs when the distance between the hilus of the spleen and the midline of the body has become decreased.

O. T. SCHULTZ.

SUBCUTANEOUS FAT NECROSIS IN THE NEW-BORN. J. BERNHEIM-KARRER, Ztschr. f. Kinderh. **55**:695, 1933.

Fifteen cases of subcutaneous fat necrosis in the new-born are recorded. Such cases are not so rare as thought. The chief etiologic factors are trauma, exposure to cold and ischemia. One instance was due to spraying with ethyl chloride. Microscopically calcification has been observed repeatedly, as well as giant cells and fatty acid crystals. The areas of necrosis disappear spontaneously, although it may take months.

JACOB KLEIN.

LICHEN RUBER PLANUS AND THE CENTROCYTES OF LIPSCHÜTZ. T. E. OLIN, Arb. a. d. path. Inst. d. Univ. Helsingfors **7**:311, 1933.

While he was studying the skin lesions of lichen ruber planus, measles, pityriasis rosea, psoriasis and German measles, Lipschütz observed hypertrophy of the microcentrum and centrioles in some of the cells. He believed that the changes were due to a centrotropic virus which caused a histiocytic tissue reaction in the corium. Olin has studied histologically twelve cases of lichen ruber planus. He found that the centrocytes of Lipschütz are free macrophages or histiocytes whose cytoplasm contains a number of large readily stained granules. They occur most frequently at the border of the epidermis and corium, particularly where the basal layer is edematous and shows regressive changes. They are also found in the inflamed corium where they are seen in the lumen of capillaries and veins. Lipschütz' theory that the granules found in these cells are the centrioles of pathologic microcentra is untenable. Olin has demonstrated in these cells a normal microcentrum and centrioles which stain characteristically differently from

the granules, which are probably condensation products, the result of various changes in the epidermis and the endothelial cells. There is no indication that they have a rôle in the etiology of the various dermatoses in which they are found.

JACOB KLEIN.

Pathologic Chemistry and Physics

THE ACCUMULATION OF IRON IN TUBERCULOUS AREAS. V. MENKIN, J. Exper. Med. **60**: 463, 1934.

Dilute solution of ferric chloride repeatedly injected into the veins of tuberculous rabbits markedly retards the development of the disease, as evidenced by a comparison of the lesions and the periods of survival of control and experimental animals. Partial immunity induced by first infection strikingly enhances the favorable effects of the iron salt in reinfected animals. Some of the experimental animals were still alive and apparently well about six months after the death of the last of the controls, which succumbed about four months after reinfection with virulent tubercle bacilli.

BLOOD AMYLASE IN EXPERIMENTAL PANCREATITIS. A. C. CLASEN, P. N. JOHNSTONE and T. G. ORR, Surg., Gynec. & Obst. **59**:756, 1934.

With 0.1 cc. of clear blood plasma and a 3 per cent solution of soluble starch as the substrate, the normal amylase content of the blood plasma of healthy dogs proved to be from 20 to 56 Davison units. Following injury of the pancreas there occurred a marked temporary rise of amylase proportionate to the degree of damage. In the presence of chronic injury normal or slightly elevated values were obtained. Cloudy swelling and necrosis about the central veins of the liver were constant concomitant findings. Determinations of the blood amylase seem to be indicated in studying possible early acute pancreatic disease.

FROM THE AUTHORS' CONCLUSIONS (W. C. HUNTER).

THE CEREBROSPINAL FLUID PRESSURE IN ARTERIAL HYPERTENSION. G. W. PICKERING, Heart **1**: 397, 1934.

The general clinical features presented by hypertensive patients with cerebrospinal fluid pressure of 250 mm. of water and over contrast strongly with those presented by patients having lower pressure. The former patients are younger, the kidneys are usually severely damaged, and the disease progresses rapidly to a fatal termination. The latter are older, the kidneys usually escape severe damage, and the disease progresses less rapidly. Every patient with a cerebrospinal fluid pressure of more than 250 mm. of water acquired albuminuric retinitis. With one exception, every patient with a lower cerebrospinal fluid pressure had either no retinal lesion or the lesions characteristic of arteriosclerotic retinitis. It is suggested that the essential difference between these two forms of retinitis is the addition, in the albuminuric type, of neuroretinal edema resulting from increased intracranial pressure. In individual patients with high blood pressure, the cerebrospinal fluid pressure has been found unaltered during headache and during acute attacks of coma and convulsions unassociated with uremia. There is a relation between high diastolic blood pressure and high cerebrospinal fluid pressure, and it is suggested that the former is one of the factors determining the latter.

AUTHOR'S SUMMARY.

THE NUCLEIC ACID CONTENT OF THE THYMUS GLAND. P. E. GRÉGOIRE and C. GRÉGOIRE, Arch. internat. de méd. expér. **9**:263, 1934.

The nucleic acid content of the thymus decreases with the rapid involution of the gland produced by roentgen irradiation. Nucleoprotease is not eliminated to any great extent during this process. It remains primarily in the medullary

portion of the gland, the cells of which are resistant to irradiation. The quantity of nucleic acid present in the gland, or liberated by it during artificial involution, is so small that the thymus can hardly be considered as a depot for nucleoprotein.

ELIZABETH MCBROOM.

ATTEMPTS TO DEMONSTRATE ARSENIC HISTOLOGICALLY IN THE SKIN. M. OPPENHEIM and P. FANTL, Arch. f. Dermat. u. Syph. **170**:488, 1934.

The authors impregnated a piece of skin with arsenic outside of the body. After inorganic arsenic they obtained arsenic trisulphide with acid hydrogen sulphide only; after arsphenamine they obtained it with neutral hydrogen sulphide in the incubator. They found greenish to egg-yolk yellow arsenic trisulphide in colloidal form, which stained the tissue diffusely, but no crystals or amorphous forms. By placing the tissue in alkaline solution of bismuth they obtained the bismuth sulphide, which is more easily seen. They could not demonstrate arsenic in the tissue of animals or human beings after these had received arsenic. They believe that the melanin in arsenical melanosis and exfoliative dermatitis contains arsenic.

This work confirms that of Tannenholz and Muir (Methods for Microchemical Demonstration of Arsenic in Tissues, ARCH. PATH. **15**:789, 1933).

S. W. BECKER.

EARLY DIAGNOSIS OF INDUSTRIAL DISEASES (METAL POISONING) BY MICROCHEMICAL BLOOD EXAMINATIONS. ERNST A. PRIBRAM, Arch. f. Gewerbepath. u. Gewerbehyg. **5**:345, 1934.

Exceedingly small quantities of metallic salts, when resorbed, are sufficient to disturb the otherwise well balanced chemical equilibrium of the blood plasma. This occurs before clinical symptoms are demonstrable and can therefore be used for early diagnosis. The method described here consists in the determination of the combining power of the blood serum, considered as colloids, for the blood crystalloids dextrose, urea and phosphorus (as phosphates). The normal serum maintains an equilibrium of these crystalloids within certain limits: from 90 to 120 mg. of dextrose, 30 mg. of urea and 3 mg. of phosphorus per hundred cubic centimeters of blood. The ability of the serum colloids to maintain the equilibrium of the crystalloids can be found by adding to the serum the physiologic quantities of urea, phosphorus and dextrose, and determining how much of each can be recovered by routine chemical methods. The loss indicates the amount combined with colloids. It has been found that normal serum combines with about 75 per cent of the added sugar, with from 30 to 50 per cent of the added urea and with from 90 to 100 per cent of the added phosphorus as sodium phosphate (Pribram, Ernst A.: *Proc. Soc. Exper. Biol. & Med.* **32**:8, 1934).

The presence of lead chloride, mercuric chloride or arsenic chloride, even in traces as small as 1:100,000 of a normal solution in 1 cc. of serum, diminishes the combining power of the serum for dextrose, urea and phosphorus. This can be demonstrated in vitro (with human serum or animal serum) as well as in vivo by injections of minute quantities of the metallic chloride. The effect of injections repeated at weekly intervals accumulates to such a degree that the combining ability of the serum colloids eventually goes to zero; i. e., they do not combine with the crystalloids. This causes a gradual loss of weight, as the serum colloids by reason of their combining power control assimilation and dissimilation ("micrometabolism," Frank Wright). The tissues—the serum is just a part of them—react in the same way, which causes a gradual increase in the value for urea, dextrose and phosphorus. Iron chloride has an opposite effect; it does not decrease but increases the combining power of the serum colloids for its crystalloids. This takes place in vitro as well as in animals studied experimentally. By repeated injections of 1 cc. of a 1:100 normal solution of iron chloride, the combining power of the serum colloids for its crystalloids can be gradually increased to 100 per cent. This means that none of the added crystalloids can be recovered. The tissues act in

the same way; the values for dextrose, urea and phosphorus go down gradually after each injection, and the weight of the animal goes up (assimilation).

The value of this method for determining the individual susceptibility to chronic poisoning is obvious. It is a convenient method for the industrial pathologist to use in determining early the noxious influence of poisons. Difficult and often unsuccessful search for the metals themselves can be replaced by search for their effect: the loss of the normal ability of the serum colloids to combine with crystalloids, to maintain their physiologic equilibrium. The serum can be used for this purpose within twenty-four hours after death.

ERNST A. PRIBRAM.

Immunology

RELAPSING FEVER IN CALIFORNIA: IMMUNITY. G. E. COLEMAN, J. Infect. Dis. **54**:1, 1934.

Immunity of variable duration is produced in mice by the injection of my three strains of relapsing fever spirochetes isolated in California. It makes no difference whether the test inoculations are made with blood taken at the same stage of the disease as that which produced the immunity or with blood taken at a different stage. There is cross-immunity between homologous spirochetes in blood specimens representing different states of the disease. Inconclusive results were obtained in experiments with serum in vitro. Delay of infection was produced with serum from mice that had recovered, but absolute immunity was conferred passively only twice. Evidence of cross-protection was obtained between two of the California strains. Judged by cross-immunity tests, the three California strains are probably identical. Spirochaeta Novyi and Spirochaeta Duttoni appear to belong to groups different from that of the California strains, though there may be some relationship between the latter and S. Novyi. It is my opinion that cross-immunity tests are wholly reliable for the differentiation of species or groups of relapsing fever spirochetes.

FROM THE AUTHOR'S CONCLUSIONS.

HEMOLYSIN AND TOXIN OF CHOLERA AND RELATED ORGANISMS. M. A. GOHAR, Zentralbl. f. Bakt. (Abt. 1) **126**:61, 1932.

Gohar studied the conditions under which hemolysin is produced by cholera vibriones and the relationship of the hemolysin to the exotoxin produced by hemolytic strains. He found that oxygen, in general, favored the production of hemolysin, independently of its effect in favoring growth. The content of toxin, as demonstrated by intradermal injection into the skins of rabbits, paralleled that of hemolysin. Treatment of the cultures by mixture with cholesterol, suprarenal tissue and brain tissue caused absorption of both the hemolysin and the toxin, whereas kaolin and neutral fats had no effect. Hemolysins from the addition of erythrocytes to the culture removed the toxin. He concludes, therefore, that the hemolysin and the exotoxin are probably identical. In the discussion he calls attention to the affinity of toxins on the whole for lipoids as shown by the congestion of the suprarenal glands from diphtheria toxin and the neurotoxic action in tetanus and botulism. He suggests that the hypothetical toxin which causes hemolysis of the red cells in pernicious anemia is probably the same toxin that causes the subacute combined degeneration of the spinal cord.

PAUL R. CANNON.

ANTIBODIES AGAINST TUBERCLE BACILLI AND A SPECIFIC THERAPY AGAINST TUBERCULOSIS. GUIDO FINZI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:76, 1934.

Lactating cows and horses were inoculated with a preparation of tubercle bacilli which was made in the following manner: Tubercle bacilli were grown for from four to six weeks on peptone glycerin broth. The bacilli formed a pellicle on the surface. The broth was pipetted off and formed the so-called exotuberculin of

Finzi. The pellicle of the bacilli remained on the walls. A 5 per cent solution of phenol in distilled water was added to the bacilli, and the suspension was kept for from four to six weeks in the incubator, after which time the bacilli were suspended in the exotuberculin. The animals were inoculated repeatedly with this antigen, first subcutaneously and later intravenously, until they developed after from six to eight months antituberculous serums which were able to neutralize two or three diagnostic doses of exotuberculin in vivo and in vitro. Serum of normal healthy oxen and horses is able to neutralize one diagnostic dose of exotuberculin. The immune serums also had high titers of specific agglutinins (1:10,000 and more) and precipitins. The milk of the immunized cows had all the aforementioned properties of the blood serum. The titer of the antibodies in the milk dropped rapidly after the inoculations were discontinued. Finzi advocates the oral use of the milk of such immunized cows for therapeutic purposes. The antibodies are not destroyed in the digestive tract and are readily absorbed.

I. DAVIDSOHN.

THE SEROLOGY OF PERTUSSIS. M. GUNDEL and W. SCHLUETER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:218, 1934.

According to Gundel and Schlueter, differentiation of the influenza and of the Bordet-Gengou bacilli is always possible. In addition to cultural differences, the bacilli of Bordet-Gengou produce local necrosis when a suspension of the live bacilli is injected intracutaneously; the influenza bacilli do not possess that property. However, the most important means of differentiation is the complement-fixation test. The antigen was prepared from a vaccine which is sold by one of the commercial houses in Germany. It contains 8,000,000,000 micro-organisms per cubic centimeter and is made from 48 hour old cultures killed in the water bath at 60 C. and preserved with 0.5 per cent phenol. The vaccine was centrifugated, suspended in 50 per cent alcohol, shaken for one hour and kept at 37 C. for twenty-four hours. Lecithin was added, and it was found that the optimum amount was 0.2 cc. for 1 cc. of the antigen. Such antigen proved highly specific and sensitive. A prolonged cultivation of Bordet-Gengou bacilli led occasionally to changes of their antigenic qualities. Strains were observed which gave with the usual technic doubtful results. They could be eliminated when the complement fixation was carried out at 0 C., at which temperature only true Bordet-Gengou bacilli reacted. Differentiation was also possible by means of adsorption of specific serums. Complement-fixation tests were positive with serums of patients suffering from pertussis in a large percentage of cases, and they were uniformly negative with serums from a large number of controls suffering from a variety of diseases. For reasons already mentioned, it is necessary to carry out two tests: one at 37 C., the other at 0 C. Of seventy serums from suspected patients, fifty-four gave positive results, three results that were doubtful, and 13 negative results. The test was rarely positive during the first two weeks, particularly in infants less than 6 months of age. In artificially immunized children the complement-fixing antibodies appeared sooner and rose more rapidly then in patients suffering from spontaneous infections. According to Gundel and Schlueter, the lack of success of previous authors with the complement-fixation test in pertussis was due primarily to the frequent selection of improper strains of bacteria for the antigens.

I. DAVIDSOHN.

GROUP-SPECIFIC SUBSTANCES IN THE BLOOD OF MICE. OTTO WUENSCHE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:293, 1934.

The study included mainly white mice (Mus musculus-albus) and to a lesser extent Japanese dancing mice (Mus Wagneri var. rotans), the common house mice (Mus musculus) and hybrids of these varieties. A pure strain of white mice was obtained by inbreeding under closely controlled conditions. Iso-agglutinins were not observed, which confirms the results of MacDowell and Hubbard. Human serums of all types agglutinated the red cells of these mice, but the agglutination by anti-A serum was uniformly more pronounced. Wuensche concludes that the

blood of mice contains the A factor. The red cells of the mice were agglutinated with marked quantitative variations by the serums of the horse, sheep, ox and pig, but the serum of mice lacked agglutinins for the red cells of the four mentioned species of animals.

I. DAVIDSOHN.

IMMUNOLOGIC REACTIONS IN MOLLUSKS. KATASHI MAKINO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:316, 1934.

Nine varieties of mollusks could be differentiated by means of precipitating serums produced in rabbits with watery extracts of the tissues of mollusks. Immune serums produced with antigens heated to 70 and 80 C. reacted specifically with the homologous antigen. The titer with the unheated antigen was usually lower. The group reactions due to zoologic interrelationships could be eliminated by means of proper absorptions. The specific precipitates differed morphologically from the nonspecific. The differentiation of the different mollusks was also successful by means of the complement-fixation test and by means of anaphylactic reactions in actively and passively sensitized guinea-pigs. Tissue extracts of mollusks when given parenterally had a toxic effect on rabbits similar to that which is generally produced in animal tissues. The rabbits lost weight rapidly. I. DAVIDSOHN.

THE PRACTICAL APPLICATION OF THE BLOOD FACTORS M AND N OF LANDSTEINER AND LEVINE. JOHANNA PUESCHEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:445, 1934.

Pueschel reports the technic which is employed at the Institute of Hygiene in Danzig for the study of the factors M and N. The immune serums were obtained by the inoculation of rabbits with properly selected red cells. Usually, 1 cc. of washed cells was injected eight or more times intravenously twice a week. Other methods of immunization yielded similar results. The titers of anti-M serums were as a rule higher than those of the anti-N serums. Schiff's method of centrifugation was used for the estimation of the agglutinin titers. For the test, the serums had to be purified by absorption with heterologous red cells, but for storage they were not purified and no preservative was added. Purified serum loses its titer rapidly. Anti-M serums are easier purified than anti-N serums. The determination of the presence of the M and N factors in a blood sample is done either directly by testing the cells with purified anti-M and anti-N serums or indirectly by treating the immune serums with the cells and by determining subsequently which agglutinins were adsorbed. The indirect method can be carried out with purified or with native serum. Pueschel found the direct method more satisfactory and more reliable, but recommends that beginners check their results with the indirect method. A study of 2,018 persons showed that the distribution of the M and N factors is similar to the findings in various regions of Germany. (M N = 50.45 per cent, M = 30.57 per cent, N = 18.98 per cent.) The procedure was employed in court for the exclusion of paternity. Thirty-two times, or in 20.5 per cent of 156 cases, such exclusion was made and accepted by the court. In 5 cases the verdict was based exclusively on the findings of the blood group study.

I. DAVIDSOHN.

COMPLEMENT FIXATION IN HERPES ZOSTER AND CHICKENPOX. OLUF THOMSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:88, 1934.

The antigen was prepared from crusts of herpes zoster and from chickenpox lesions. The results of complement-fixation tests with the serums of eighty-eight patients with herpes zoster and with fourteen patients with chickenpox were entirely negative. Thomsen calls attention to the recent report of Brain of England who obtained positive results with an antigen prepared from the vesicular fluid, and negative results when crusts were used as antigen. Brain's work confirms some previously expressed opinions of a close serologic relationship between the two diseases.

I. DAVIDSOHN.

THE ACTION OF PNEUMOCOCCUS ANTISERUM. L. OELRICHS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:124, 1934.

A pneumococcic antiserum which protected mice was equally efficacious in rabbits. The efficacy, which was determined by the effect on the bacteremia, the leukocytosis, the temperature and the mortality, was influenced mainly by the interval between the infection and the administration of the serum. The individual factors of resistance to the infection and of response to the serum were next in importance. A precipitous drop in the number of leukocytes and a bacteremia were the most ominous signs. Properly timed and repeated administration of the therapeutic serum was able to save animals when either leukopenia or bacteremia set in, but the serum was of no avail when both complications were present. The therapeutic effect of the serum was of a general nature, leading to a rapid elimination of the bacteria, as could be determined by bacterial counts in blood cultures. Local lesions were not influenced.

I. DAVIDSOHN.

THE RETICULO-ENDOTHELIAL SYSTEM AND THE NATURAL IMMUNITY OF WHITE MICE AGAINST BACILLUS PSEUDO-ANTHRACIS. C. RUSSEFF, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:141, 1934.

Splenectomy or blocking of the reticulo-endothelial system with india ink or with sublethal doses of Bacillus pseudo-anthracis protected mice against otherwise uniformly fatal doses of B. pseudo-anthracis. Russeff assumes that the death of the unprotected animals was due to liberation of harmful products during the destruction of the bacteria by the reticulo-endothelial cells. Elimination of a major part of the reticulo-endothelial system by blockade or by splenectomy removed the possibility of damage. Under normal circumstances the number of bacteria which are acted on by the reticulo-endothelial system is very small.

I. DAVIDSOHN.

THE ORGAN SPECIFICITY OF THE LENS. WALTER ARNOLD, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:154, 1934.

Boiling of the lens tissue decreased its ability to fix complement. Immune serums produced with the boiled lens reacted more strongly with the homologous antigen than with the native lens. Alcoholic extracts of lens tissue reacted less strongly in the complement-fixation tests than did the native lens. The immune serums produced in rabbits by inoculation with alcoholic extracts of lenses mixed with hog serum had, as a rule, the properties of lipoid antiserums, but occasional antiserums obtained after a shorter series of inoculations had organ-specific properties. When the complement-fixation test was done at icebox temperature even the lipoid antiserums occasionally exhibited organ-specific properties.

I. DAVIDSOHN.

Tumors

A MIXED TUMOUR (RENAL BLASTOCYTOMA) OF THE PAROVARIUM. J. S. YOUNG and C. G. LOWRY, J. Path. & Bact. **39**:179, 1934.

A mixed tumor (renal blastocytoma) of the right parovarium in a woman of 46 is described. It was associated with a congenital elongation of the right fallopian tube.

FROM THE AUTHORS' SUMMARY.

PARASITES AND TUMOR GROWTH. R. HOEPPLI, Chinese M. J. **47**:1075, 1933.

According to Hoeppli, while it is possible in experimental production of tumors to determine the various factors on the part of the parasite as well as of the host which play a rôle in the development of the tumors, one has to realize that there remains always an additional unknown factor X which is specific and must com-

bine with the other factors in order to change the cells biologically in such a way that they acquire the well known characteristics of malignant tumor cells. Nothing is known about this X which in the deepest sense is the real cause for the development of malignant neoplasms. Parasites represent only a relative factor and act only indirectly but in this way undoubtedly play a rôle in the etiology of certain tumors. The article includes a complete bibliography on parasites and tumor growth.

A Brain Tumor in the Virchow-Robin Sheaths. I. Nicolesco, E. Craciun, I. Bazgan and T. Hornet, Ann. d'anat. path. **10**:287, 1933.

A cellular glioma of an unusual character is described. It seems to have developed in a perivascular manner in the Virchow-Robin spaces; at the periphery of the tumor it was seen to spread and invade the adjacent brain tissue only along these spaces. The tumor was located in the white matter of the right cerebral hemisphere and had produced a gradually developing hemiplegia. The cell type was a small undifferentiated dark nucleated cell with indistinct cytoplasm, but in places differentiated forms of astrocyte type could be seen.

Perry J. Melnick.

A New Form of Medulloblastoma—Medullomyoblastoma. G. Marinesco and M. Goldstein, Ann. d'anat. path. **10**:513, 1933.

In a girl 5 years old a tumor of the vermis of the cerebellum was found at autopsy, of an unusual character. In general the tumor had the histologic character of a medulloblastoma. In addition there was a large admixture of striated muscle fibers. The striated muscle fibers were found in various stages of embryonic development, up to fully differentiated muscle fibers. In a discussion of the histogenesis no definite conclusions are arrived at except that an embryonic factor in its development is apparent.

Perry J. Melnick.

Pulmonary Lesions Following Stenosing Cancer of the Main Bronchi. M. Chiray, G. Albot and R. Jane, Ann. d'anat. path. **10**:527, 1933.

The secondary lung changes in two cases of stenosing carcinoma of the main bronchi are described. These include chronic bronchitis, bronchiectasis, metaplasia of the bronchial epithelium, chronic and subacute pneumonia, atelectasis, hemothorax, etc. These changes tend to become the prominent ones both clinically and roentgenologically, and are prone to obscure the carcinoma itself.

Perry J. Melnick.

Lacunar Meningoblastoma of the Spinal Meninges. L. Cornil and M. Mosinger, Ann. d'anat. path. **10**:725, 1933.

The authors distinguish two forms of meningoblastoma, an epithelial type and a mesenchymatous type (Oberling, Roussy and Cornil). Of the latter there are three forms: (1) fascicular, (2) concentric and (3) reticular. The last form resembles schwannoma. They report a case which can be classified as a subvariety of the reticular form, a lacunar meningoblastoma. The microscopic picture was characteristic in that the fibrillar network of the tumor was loosened in a regular fashion, forming large regular spaces or lacunae. In some fields the tumor resembled an ordinary fibrillar meningoblastoma.

Perry J. Melnick.

Sarcoma of the Pancreas. C. Grandclaude, M. Lambret and J. Driessens, Ann. d'anat. path. **10**:861, 1933.

Sarcoma of the pancreas is rare. About forty cases have been reported. Three anatomic forms have been described: (1) solitary, (2) nodular and (3) cystic.

Microscopically three types are known: (1) lymphosarcoma, (2) spindle cell sarcoma and (3) angiosarcoma. The authors describe a cystic angioleiomyosarcoma in a man 55 years old.

PERRY J. MELNICK.

DETECTION OF COAL TAR AND ITS DERIVATIVES IN THE LUNG. H. R. KANITZ, Virchows Arch. f. path. Anat. **292**:175, 1934.

Some writers have ascribed the increased incidence of carcinoma of the lung noted in recent years to the inhalation of dust containing asphalt and coal tar and their derivatives, substances that are known to have a carcinogenic action. This theory could be put to the proof only if some method for accurately determining the presence of tar and its derivatives were available. In the experiments reported by Kanitz weighed quantities of the substances studied were introduced into the trachea at its bifurcation through a tracheotomy incision. The animals were killed at varying intervals, and the material present in the lung was determined by methods that are accurate to within 0.01 mg. The substances used were the coal tar derivatives anthracene, carbazole and acridine, anthracene oil, and thin and thick distilled tar. Anthracene, carbazole and acridine disappeared from the lung more quickly than did the tars. They are removed chiefly through the trachea by respiration, although acridine appears also to undergo chemical change in the lung. The tars remain in the lung for longer periods because of their viscid character. They are broken down by oxidation, apparently after union with the fats present in the lung. Tar could not be detected in the spleen or liver, even when relatively large quantities had been introduced into the trachea.

O. T. SCHULTZ.

THE INNERVATION OF TUMORS. H. OERTEL, Virchows Arch. f. path. Anat. **292**:249, 1934.

Continuation of previously published work has led to confirmation of the view that all tumors have nerve fibrils. The latter are present throughout the tumor, bear a relationship to the tumor elements, and take part in the growth of the tumor. In tumor growth, as in normal and pathologic growth, the tissue grows as a whole.

O. T. SCHULTZ.

ACTION OF ENZYMES OF MALIGNANT TUMORS ON ELASTIC TISSUE. D. M. TICHOMIROW, Virchows Arch. f. path. Anat. **292**:310, 1934.

In experiments in vitro, extracts of sarcoma were found to have a strong digestive action on the elastic tissue of the arterial wall. The optimum hydrogen ion concentrations lay between p_H 6.813 and 7.168, and between p_H 7.381 and 7.731. Extracts of carcinoma had a much less marked action on elastic tissue under identical conditions; the optimum reaction lay between p_H 7.168 and 7.381. The tendency of sarcoma to metastasize by way of the blood stream is explained by the strong action of the enzymes of sarcoma on the walls of blood vessels.

O. T. SCHULTZ.

COMPARATIVE MEASUREMENTS OF NUCLEUS AND NUCLEOLUS WITH SPECIAL REFERENCE TO CANCER. E. HAUMEDER, Ztschr. f. Krebsforsch. **40**:105, 1933.

A comparative study was made by Haumeder of the relations in size of nucleolus to nucleus both in normal tissues and in cancer. With both there was much greater variation in cancerous tissues. The average values of both were increased here, but this was more conspicuous with the nucleolus, which frequently showed great increase of size even with nuclei which were but little enlarged. The average ratio of area of nucleolus to that of nucleus in normal cells varied from 1:13 to 1:45 and over. In cancer cells it ranged from 1:5 to 1:7. Haumeder believes that this alteration is sufficiently definite and constant to be of diagnostic value.

H. E. EGGERS.

THE CHARACTERISTICS OF FACIAL CANCER. E. PALJTSCHEWSKY, Ztschr. f. Krebsforsch. **40**:159, 1933.

This report is based on the study of 247 cases of facial cancer as observed at the Ukrainian Roentgen-Radiological Institute. All were studied histologically. The greater part of the cases occurred in peasants; next came laborers in industries involving chemical or thermal irritation. Two-thirds were in males. As regards types of cancer, 49 were of the basal cell type; these occurred mostly during the middle period of age. Nonkeratinizing squamous cell carcinomas occurred usually at an earlier age, and formed 35 per cent of the cases, while keratinizing tumors of this type were observed principally at advanced ages, and constituted 16 per cent. The basal cell tumors were located principally in regions rich in sebaceous glands, while the akeratinized squamous cell cancers were situated predominantly around the mucous orifices. With all types, there was a relation to physiologic cutaneous atrophy. With the spinocellular cancers there were usually preceding changes in the epidermis, while with the basal cell variety these antecedent changes were more deeply located, and involved either sebaceous glands or hair follicles, or were of the type of comedones, acne or nevus.

H. E. EGGERS.

THE NATURE OF VIRILIZING OVARIAN TUMORS. HILDING BERGSTRAND, Acta path. et microbiol. Scandinav., supp. 16, p. 31, 1933.

Heterologous secondary sexual characteristics may be classified into three symptom complexes: (1) hirsutism in sexually mature women who have benign or malignant ovarian tumors or benign suprarenal tumors; (2) precocious puberty combined with hirsutism due to malignant suprarenal tumors; (3) congenital feminine pseudohermaphroditism with enlarged suprarenal glands.

In a previous report Bergstrand described six cases of ovarian tumor with hirsutism due to granulosa cell tumors (arrhenoblastoma). These arise from the masculine embryonic remnants in the rete ovarii. Virilizing hormones are also found in the corpus luteum and in the urine of pregnant and nonpregnant women. In most instances the virilizing tumors arise from ovarian tissues in various stages of development.

JACOB KLEIN.

THIRTY-FIVE CASES OF CEREBELLOPONTILE ANGLE TUMORS. P. WINDFELD, Acta path. et microbiol. Scandinav. **11**:146, 1934.

The present material does not permit the drawing of any conclusions as to the ectodermal or mesodermal nature of the neurinomas and meningiomas. It shows, however, that these two forms of tumor, besides possessing a peculiar individualness, have several histologic points of resemblance, and that the site or the macroscopic appearance of the tumor does not permit determination of whether one has to deal with one form or the other. Thus, our material is not supportive of Folke Henschen's supposition that the neurinomas originate in the distal intratemporal portion of the eighth cranial nerve, but points rather in the direction of a histogenetic relationship between neurinomas and meningiomas.

THE GENETIC FACTOR IN BENIGN TAR TUMORS IN MICE. L. KREYBERG, Acta path. et microbiol. Scandinav. **11**:174, 1934.

In mice a genetic factor has been proved to influence the reaction to tar painting which leads to formation of benign tumors of the skin. Consequently experiments which aim at illustrating the influence of various factors on the development of tar tumors must be conducted with material the genetic constitution of which is known.

Technical

THE TRYPTOPHANE TEST IN TUBERCULOUS MENINGITIS. MILTON B. ROSENBLATT, Am. Rev. Tuberc. **29**:668, 1934.

The tryptophan test is made by adding to from 2 to 3 cc. of spinal fluid from 15 to 18 cc. of concentrated hydrochloric acid and 2 or 3 drops of 2 per cent solution of formaldehyde; then the tube is inverted a few times and allowed to stand for from four to five minutes, when from 1 to 2 cc. of a 0.06 per cent solution of sodium nitrite is layered over it. A positive reaction is indicated by a delicate violet ring in a few minutes. A positive test is strongly suggestive but not pathognomonic of tuberculous meningitis. Hemorrhagic, purulent and xanthochromic fluids give false positive reactions, which are usually distinguishable from those in tuberculous meningitis by the purplish color. Indistinguishable positive reactions may be obtained with clear spinal fluids in which the protein is increased.

H. J. CORPER.

THE PREPARATION OF COLLOIDAL GOLD. D. P. BOROWSKAJA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:178, 1934.

The directions are as follows: To 95 cc. of distilled water add 1 cc. of a 1 per cent solution of gold chloride. Heat the mixture to from 90 to 95 C., add 5 cc. of a 1 per cent solution of sodium citrate and bring to the boiling point, letting the mixture boil for from one to three minutes. Simple distilled water and plain glass proved satisfactory. The product proved sufficiently sensitive with pathologic cerebrospinal fluids, and very stable (for at least two to three months).

I. DAVIDSOHN.

THE CABITTO TEST FOR ALBUMIN IN THE CEREBROSPINAL FLUID. E. WEICHHERZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:334, 1934.

In 1932 Cabitto published a new test for albumin in the cerebrospinal fluid. The reagent is a 0.5 per cent solution of phosphomolybdic acid, which is filtered after preparation and kept in a dark bottle. To 1 cc. of cerebrospinal fluid, 0.2 cc. of the reagent is added. The test tube is shaken, and the result is read. Weichherz tried the test in 346 cases, using half of the original quantities and found it more sensitive and specific than the Pandy, the Ross-Jones and the Nonne-Apelt-Schum tests.

I. DAVIDSOHN.

Medicolegal Pathology

SOME MEDICOLEGAL ASPECTS OF ISO-AGGLUTININS. H. A. HEISE, Am. J. Clin. Path. **4**:400, 1934.

Heise presents four medicolegal cases in which he applied the blood grouping method. In the first case, dried blood which had been exposed to the elements was successfully grouped; in the second and third cases the blood groups of dried seminal stains were determined, and in the fourth an exclusion of the alleged paternity was obtained in a case of bastardy, one of the few in the United States in which the results of the blood grouping tests were introduced into the court as evidence. In the last-named case, Heise found that the accused man belonged to group O, the woman to group A and the baby to group B. The man had evidently been falsely accused. The jury ignored this evidence and found the man guilty on account of a fancied resemblance between the infant and the accused man's father. Fortunately, the judge was not deceived, found the judgment to be against the evidence, and granted a new trial. The new trial was never held, as the child developed distinct negroid facial characteristics, although the mother and the supposed father are white.

STATUS LYMPHATICUS. D. SYMMERS, Am. J. Surg. **26**:1, 1934.

Status lymphaticus may be defined as a combination of hereditary constitutional anomalies, entering into which, among other things, are certain peculiarities of configuration, with preservation or even hyperplasia of the thymus at an age when involution is to be expected, hyperplasia of the lymphoid cells in the spleen, intestines and elsewhere, changes in the distribution of hair and hypoplasia of the vascular system. Status lymphaticus is compatible with prolonged life, but occasionally it is associated with sudden death on trivial provocation due, apparently, to anaphylaxis, or to spontaneous rupture following increased intracranial pressure acting on a congenitally hypoplastic vessel. Status lymphaticus is encountered in about 6.2 per cent of all autopsies. It occurs in males six times oftener than in females.

FROM THE AUTHOR'S SUMMARY.

UNUSUAL FATAL STAB WOUNDS OF HEAD AND NECK. M. HELPERN, Am. J. Surg. **26**:53, 1934.

Four examples of fatal penetrating stab wounds of the head and neck are described. In three instances, the weapon used was probably an ice-pick, and in one, a thin-bladed knife. In two of the cases, the small surface wounds on the scalp were not detected clinically, being concealed by the hair, and in one case a small wound on the forehead was mistaken for a superficial laceration. In two examples of stab wounds of the neck involving the left common carotid artery, a mural thrombus developed in the arterial wound and gave rise to an embolus which lodged in the left middle cerebral artery, causing death from cerebral infarction. A fatal wound of the neck, left innominate vein and arch of the aorta resulted in an arteriovenous aneurysm which dissected the anterior mediastinum and ruptured into the left pleural cavity, where a massive hemothorax was formed.

FROM THE AUTHOR'S SUMMARY.

TRAUMATIC PEPTIC ULCER. G. B. EUSTERMAN and J. G. MAYO, Am. J. Surg. **26**:74, 1934.

There is apparently adequate evidence to justify the contention that under exceptional circumstances a chronic ulcer of the stomach can have its origin in an external (nonpenetrating) trauma to the epigastric region. In one case, fairly characteristic symptoms of a hemorrhagic gastric ulcer developed following a severe blow to the left epigastric region. Five months after the injury, roentgenoscopy confirmed the presence of a penetrating ulcer near the lesser curvature. Following hospitalization of the patient and intensive medical treatment, the lesion disappeared. In any case in which a plaintiff claims that a gastric or a duodenal ulcer followed external trauma, the physician whose opinion is sought should see that the four postulates of Liniger and Molineus are satisfied. A second cause of ulcer is chronic trauma from within the body. Ulcer of this nature is most commonly secondary to congenital (nontraumatic) diaphragmatic hernia. Foreign bodies in the stomach may also cause ulcer. Such secondary lesions are not as a rule indurated, and following reduction of the hernia or removal of the foreign body, tend to heal readily.

FROM THE AUTHORS' SUMMARY AND CONCLUSIONS.

TRAUMATIC INTRACRANIAL HEMORRHAGES. TIMOTHY LEARY, Am. J. Surg. **26**:133, 1934.

Typical cases of intracranial extracerebral hemorrhages which escaped diagnosis are presented. The opportunity for obtaining exact information by a relatively minor operation, the exploratory bur hole procedure, which Munro and his associates at the Boston City Hospital have developed, should serve to clear up some of the mysteries in this neurologic field. When it is remembered that the only treatment of intracranial extracerebral hemorrhages is operative, and that the

results of successful operation are brilliant, the necessity of using every means to perfect the diagnosis of postraumatic cerebral disturbance is obvious.

From the Author's Summary.

Socalled Spontaneous Subarachnoid Hemorrhage. W. D. Ayer, Am. J. Surg. **26**:143, 1934.

Spontaneous subarachnoid hemorrhage is usually the result of a rupture in an aneurysm of the circle of Willis. The qualification "spontaneous" is objected to as unsatisfactory and confusing. The etiology of aneurysms of the circle of Willis is difficult to determine. Those occurring without inflammatory or degenerative vascular changes usually have been considered congenital. It is suggested that many may have been mechanically acquired. The circle of Willis, because of its peculiar arrangement and structure, is subjected to unusual stress and may be, in consequence, susceptible to the mechanical formation of aneurysms. A severe cerebral trauma may be a factor. Exertion immediately preceding a rupture may be a cause of the rupture and hence has medicolegal importance. Approximately 50 per cent of the subjects recover. The aneurysms may partially rupture and heal to rupture again, or they may become thrombosed and absorb completely. Two per cent of sudden deaths are due to ruptured aneurysms.

From the Author's Conclusions.

Medicolegal Aspect of Deaths with Chloroform or Ether. A. O. Gettler, Am. J. Surg. **26**:168, 1934.

A dog killed by chloroform administered in excessive amounts had 551.5 mg. of this anesthetic in 1,000 Gm. of brain. Two animals while fully anesthetized had 270 mg. and 284.6 mg. of chloroform in 1,000 Gm. of brain. When an animal was in the stage of recovery, the chloroform content of the brain decreased very rapidly during the first thirty-four minutes, so that there was only 51.3 mg. left in 1,000 Gm. of brain. From then on, the chloroform content decreased more slowly. After one hundred and ninety minutes there was only 0.16 mg. of the anesthetic present in 1,000 Gm. of brain. After forty minutes the animals appeared normal. At this time they probably had about 45 mg. of chloroform in 1,000 Gm. of brain. From fifteen to thirty minutes after the animals seemed normal, there was from 30 to 35 mg. of chloroform in 1,000 Gm. of brain. In the stage of recovery, the lungs contained much less chloroform than the brain. Human beings during anesthesia were found to have between 120 and 182 mg. of chloroform in 1,000 Gm. of brain. In cases of suicide and homicide by chloroform, 1,000 Gm. of brain was found to contain from 372 to 480 mg. of this drug. A method for isolating small amounts of ethyl ether from human tissues is described. Amounts as small as 0.06 cc. in 500 Gm. of tissue were isolated and measured. From tissues containing between 0.3 and 0.9 cc. of ether in 500 Gm., the recovery was 83.6 per cent (average). The amount recovered is fairly proportionate and can be used for estimating the amount originally present in the tissues. The microboiling point served to identify the isolated liquid as ethyl ether.

From the Author's Summary.

Subdural Hemorrhages. T. Leary, J. A. M. A. **103**:897, 1934.

Subdural hemorrhage is not rare but is largely overlooked. The source in cases unaccompanied by fracture of the skull is usually a ruptured bridging vein or a ruptured arachnoidal vein. Alcoholism favors the occurrence. The hemorrhage tends to be unilateral. The chronicity in many cases is due to the inability of the relatively avascular dura to organize the subdural clot efficiently and to the resulting repeated secondary hemorrhages. Inflammatory reactions in connection with repairing lesions are incidental or accidental. The only practical method of curing the condition is that of operative intervention.

From the Author's Conclusions.

Society Transactions

BUFFALO PATHOLOGICAL SOCIETY

Regular Meeting, Nov. 3, 1934

KORNEL TERPLAN, Chairman

HEMOGLOBINURIA AND UREMIA FROM QUININE IN A PREGNANT WOMAN. KORNEL TERPLAN and CARL T. JAVERT.

Hemoglobinuria and hemoglobinemia following medication with quinine in early pregnancy and in malarial therapy have been reported in a few instances by Seitz, Petri and Zoeller in the continental literature. To our knowledge, hemoglobinuric damage of the kidney with uremia resulting from the use of quinine as an abortifacient has not been reported in the American literature.

The case of a 41 year old white mother of 3 children, with normal puerperia, is reported. On admission to the hospital the patient complained of persistent vomiting, anorexia, prostration and vaginal bleeding of several weeks' duration. A detailed history was refused, but was obtained post mortem from the immediate family. A pregnancy of approximately three months' duration had been terminated, and certain drugs had been taken. These were: liquor sedans (containing black haw, hydrastis and Jamaica dogwood), 20 tablets of quinine (5 grains [0.3 Gm.]), 6 bile salt tablets and 24 black pills, which on analysis contained ferrous carbonate (apiol had been suspected). Symptoms began after the ingestion of these drugs. Hospitalization was refused until about three weeks had elapsed.

She was a pale obese woman. The temperature and the pulse and respiratory rates were normal. The conjunctivae were anemic. There were brown crusts on the tongue. Colostrum was present in the breasts. There were no cardiac bruits. The blood pressure was 110 systolic and 66 diastolic. The abdomen was soft. The uterus was slightly enlarged, and the cervical canal was open, with slight vaginal bleeding. There was pitting edema of the ankles, and the back and buttocks showed a maculopapular eruption.

The urine was clear. Specific gravity was 1.015; albumin was present (2 plus). No sugar was found. The red cell count was 1,830,000 per cubic millimeter; hemoglobin, 36 per cent (Newcomer). The white cell count was 19,800, with polymorphonuclears, 96 per cent. The Wassermann reaction of the blood was negative. Chemical analysis of the blood showed in milligrams per hundred cubic centimeters: urea 344, dextrose 262, chlorides 479, calcium 6.6, phosphorus 20.4, cholesterol 221, creatinine 16.2 and uric acid 20.9. Analysis of the blood serum showed: nonprotein nitrogen 297, albumin 3.4 and globulin 1.7. The carbon dioxide-combining power in millimeters of mercury was 9. The result of the van den Bergh test was 0.6 unit.

Several samples of urine were negative for mercuric bichloride. Despite a daily ingestion of from 3,000 to 4,000 cc. of saline solution and 5 per cent dextrose, distinct oliguria was present, only 300 to 400 cc. being passed daily. A blood transfusion of 450 cc. was given. During the final days of the patient's life, the urine became loaded with red blood cells. The patient expired six days after admission.

Examination five hours after death showed: large kidneys weighing 260 Gm. each; distention of the capsules; chocolate brown surfaces, with innumerable blue-black dots; markedly swollen cortices; many reddish-brown streaks in the pyramids suggesting hemoglobinemic or methemoglobinemic infarcts; contracted bladder with turbid bloody content; a yellowish-brown liver with marked edema; definite ammoniacal odor of gastro-intestinal mucosa; necrotic placental remnants in the uterus; dark red fluid blood in the large veins; anemia and edema of all organs

including the brain; petechial hemorrhages in the epicardium, cerebrum and gastrointestinal mucosa; recent lobular pneumonia.

Histologically, the kidneys showed marked distention of some of the convoluted and collecting tubules, which contained reddish-brown granular masses of hemoglobin; other tubules contained leukocytic and necrotic epithelial casts. Bowman's capsules were also distended but contained no hemoglobin; the glomeruli appeared compressed. Comparatively few preserved erythrocytes were seen without hemolysis. Epithelial cells of some convoluted tubules, especially in the subcapsular zone, gave a positive iron reaction (hemosiderin), but the hemoglobin content of the tubules did not show this reaction. Marked interstitial inflammation surrounded the collecting tubules. The liver showed distinct selective hemosiderosis of the Kupffer cells; this was also demonstrated in the reticulum cells of the splenic pulp.

Quinine, not less than 100 grains (6.47 Gm.) in amount, was believed to be the chief etiologic factor. Three grains (0.2 Gm.) was recovered from the liver (analyzed by Dr. Edward J. Powers, chemist, Department of Health). No trace of the other drugs was found.

GLYCOSURIA OF PREGNANT AND LACTATING WOMEN. HENRY J. BROCK and ROGER S. HUBBARD.

Separate portions of urine were treated with Bacterium coli and Bacillus proteus, and the residual reducing power was determined by Sumner's dinitrosalicylic acid method. This technic gave separate readings for the residual (noncarbohydrate) reducing substances of urine, the residual reducing substances plus lactose, and the total reducing compounds, including all of the fermentable sugars. From these figures it was possible to calculate separately the lactose and the nonlactose sugar content of urine. (Xylose is fermented by Bact. coli, and, like lactose, is not fermented by the strain of B. proteus used. Xylose, therefore, would be determined together with lactose in these experiments. The existence of xylosuria seems improbable; the sugar of pentosuria, formerly thought to be xylose, has been shown to be xyloketose. This is not fermented by Bact. coli, and therefore would not interfere with the analysis for lactose.)

The method was capable of demonstrating lactose in concentrations as low as 5 to 10 mg. per hundred cubic centimeters of urine.

The method was applied to the study of urine from pregnant and lactating women and to that of urine from a control group between the ages of 18 and 45 years. A summary of the results is given in the table.

Average Values of Lactose and Dextrose in Urine of Pregnant and of Lactating Women

Group	Number	Lactose, Mg. per 100 Cc.	Dextrose, Mg. per 100 Cc.
Control	24	2.5	19.8
Lactating	11	148.0	37.6
Pregnant	42	23.0	72.4

The urine of 3 of the normal women showed a lactosuria which may have been significant (concentrations between 10 and 14 mg. per hundred cubic centimeters), but 75 per cent of the samples from this group gave results which were negative (less than 5 mg. per hundred cubic centimeters). The lactosuria, when present, was probably alimentary in origin.

The urine of 10 of the lactating women was studied within eleven days after delivery of their babies. The urine from all but 1 of these contained significant amounts of lactose (more than 10 mg. per hundred cubic centimeters), and the remaining urine, obtained on the eleventh day after delivery, apparently contained 9 mg. per hundred cubic centimeters. The urine of a woman who had been

nursing her child four months was studied. The specimen contained 57 mg. of lactose per hundred cubic centimeters. It seems almost certain that this marked lactosuria resulted from an overflow of active mammary glands.

Seventy-one per cent of the samples of urine obtained from pregnant women contained more than 10 mg. per hundred cubic centimeters of lactose, and only 6 of the 42 specimens studied gave results which were unquestionably negative by the method used—less than 5 mg. per hundred cubic centimeters. Positive and negative values were obtained more or less at random throughout pregnancy, although the highest values were found in the three months immediately preceding delivery. We believe that this lactosuria results from an increased activity of the mammary glands.

Other sugars, which were probably largely or wholly dextrose, were present in much greater concentration in the urine of pregnant women than in that from either the control or the lactating group. There did not seem to be any parallelism between the lactose and dextrose contents of the individual specimens. The average values of the dextrose content of the urine from lactating women were higher than those of the control series. The distribution of the individual values in the two groups was almost identical, however, and it seems probable that little emphasis should be placed on an interpretation of the difference between the average figures.

SICKLE CELL ANEMIA: TWO UNUSUAL CASES, WITH AUTOPSY. C. SCOTT RYERSON and KORNEL TERPLAN.

These cases are presented for the following reasons. The first was one of an unusually severe and acute form. Death could be attributed only to the acute anemia. There were no evidences of prolonged destruction of blood. The second case represented a heretofore unknown complication responsible for death—an acute severe dystrophy of the liver.

CASE 1.—Previously in good health, a 3½ year old Negro died after an illness of two days' duration. The red cell count was 1,280,000; the hemoglobin was 18 per cent. Seventy-five per cent of the erythrocytes showed typical "sickling" in an eighteen hour hanging drop preparation. The total leukocyte count was 21,700.

A huge spleen (425 Gm.) and an extreme generalized anemia were the sole findings of note at autopsy.

Microscopic examination of the spleen showed a marked predominance of the red pulp with corresponding reduction in the lymphatic tissue. The meshes of the reticulum were stuffed with sickle-shaped corpuscles, and in places actual hemorrhage, but only focal hemosiderin pigmentation, was seen. The sinusoids were markedly compressed, and their endothelial linings were occasionally interrupted by exuding sickle cells. In the light of the modern conception of the splenic circulation, it was thought impossible to identify openings in the walls of the sinusoids as congenital defects, described by Rich as being pathognomonic of this disease.

The liver showed a few huge Kupffer cells overloaded with sickle-shaped erythrocytes, but no pigment. The epithelial cells of the convoluted tubules showed iron-positive granules, and the bone marrow a conspicuous erythropoietic activity.

CASE 2.—A Negro girl of 11 years had numerous respiratory infections and frequent bouts of abdominal pain associated with vomiting. At the time of admission to the hospital her complaints were abdominal pain, vomiting, cough and pain in the left side of the chest. She appeared extremely ill. A definite icterus, a generalized enlargement of the lymph nodes, evidences of consolidation in the left side of the chest, an enlarged heart, a tender, enlarged liver, a stiff neck, and a positive Kernig sign comprised the notable physical findings. The red blood cell count was 1,750,000; the hemoglobin, 35 per cent. The white blood cell count was 69,000, 92 per cent of which were immature neutrophils. Fourteen nucleated red cells per hundred white cells were enumerated.

After a critical ten day period, the chest cleared, the liver became smaller, and the jaundice disappeared. The child seemed well except for the severe anemia, which persisted in spite of repeated transfusions. However, in about two weeks she was returned to bed because of steadily increasing anemia, a jaundice which finally became profound, persistent epistaxis and an enlarged, tender liver. She died two months following admission.

Autopsy revealed extreme jaundice and marked anemia of the entire integument. The liver was normal in size, but softened in consistency. On section the markings were entirely obscured. The spleen was represented by an oblong fibrous structure, weighing only 2 Gm. The kidneys were swollen. The heart was dilated.

Microscopically, the liver showed complete loss of acinous markings and severe regressive changes varying from fatty droplets to loss of nuclei and complete necrosis. A tremendous amount of bile pigment and hemosiderin pigment was seen in hepatic and interstitial cells; especially conspicuous was the unusually large number of Kupffer cells stuffed with sickle-shaped erythrocytes. The capillaries between the degenerated liver cell cords were markedly dilated. This picture closely simulated that recognized as the "red stage" of acute dystrophy of the liver.

Sections of the spleen showed a shrunken capsule, closely packed, hyalinized trabeculae containing numerous iron and calcium incrustations, and a small area of fibrosed red pulp with obscured sinusoids. The kidneys showed a marked hemosiderosis with icteric nephrosis.

Smears of the bone marrow demonstrated numerous myelocytes and abundant nucleated red cells, some of which were sickle-shaped.

NEW YORK PATHOLOGICAL SOCIETY AND NEW YORK ACADEMY OF MEDICINE, SECTION OF SURGERY

Joint Meeting, Nov. 22, 1934

WILLIAM C. VON GLAHN, *President, in the Chair*

SYMPOSIUM ON NONMALIGNANT TUMORS OF BONE

PATHOLOGIC ASPECTS. CHARLES F. GESCHICKTER, Baltimore (by invitation).

From the standpoint of histogenesis, benign tumors of bone are conveniently studied in relation to the bones of the skull, which are composed of both intracartilaginous and intramembranous bones. Osteochondroma may occur at the base of the skull from the portion of the occipital bone preformed in cartilage. Similar growths are found in the long bones more frequently. Benign osteoma occurs most commonly in the membranous bones of the skulls of children. In contrast to osteochondroma, which shows a transition from fibrous tissue to cartilage to bone, the osteoma shows ossification directly from fibrous tissue.

Giant cell tumor and its healing phase of benign bone cyst are usually found in intracartilaginous bone. It represents an exaggeration or an end-stage of the resorption of bone by giant cell osteoclasts. Areas of bone resorption in the membranous bones of the skull are rarely true bone cysts, being usually the result of decalcification by direct pressure from without or associated with parathyroid disturbances.

Myositis ossificans must be considered in relation to the bones of the extremities. Under the microscope, posttraumatic formations of new bone are extremely difficult to distinguish from sarcoma, and therefore clinical and roentgenologic evidence is of more importance than that from pathologic sources.

DISCUSSION

JAMES EWING: Dr. Geschickter has covered a wide field in a most entertaining way. He has endeavored, I think very properly, to distinguish in these types of bone tumors the differences depending on the embryonal history of the bones involved, the intracartilaginous and the intramembranous, and I think he has succeeded in establishing some important differences. There are curious features about many tumors of intramembranous bones which are not well represented in tumors of the other bones, but I am afraid the distinctions will not be without exception. Last week Dr. Jaffe brought me 5 specimens of tumors of bone, including 2 of the small bones of the foot and 1 of the tibia, which showed the essential features of rapidly ossifying osteoma, which Dr. Geschickter speaks of as intramembranous osteoma. Then, I am inclined to think that the exclusive appearance of cartilage under certain circumstances is not invariable, because in certain instances intramedullary osteochondroma has seemed to do everything that a tumor of the intramembranous type does. Nevertheless, in general, I think that Dr. Geschickter has emphasized an important point in making these distinctions, which are usually observed.

He warns pathologists about myositis ossificans. Most experienced pathologists and clinicians approach the subject with caution. There is no doubt that one must regard this process as presenting almost every degree of activity from the wholly benign, slowly growing process to an actively growing tumor reaching a malignant stage immediately or after a period of months or years. Recently I studied a case of tumor of the tibia with the history and roentgenologic appearance of myositis ossificans lasting for two or three years, which lately had shown signs of activity. After aspiration biopsy the leg was amputated, and in various areas of an old myositis ossificans there were active foci of fully malignant osteogenic sarcoma. I can recall many cases in which the balance between a benign process which would have gone on to slow healing and a malignant process was extremely narrow. I agree that the pathologist ought to approach myositis ossificans with great caution and express his opinion to the clinician with much reserve. How much more cautious should the surgeon be in proceeding to radical measures!

Dr. Geschickter brought up the subject of giant cell tumors, but he seemed to me to dodge this subject to some extent. No doubt one could spend an entire evening discussing it. The tendency is for giant cell tumors to undergo regression and cyst formation, and if the patients are so fortunate as to have the tumors hastened in this direction by roentgen treatment, this outcome will be the rule.

I think one should frankly accept the relation of disturbed calcium metabolism to Recklinghausen's disease, osteitis fibrosa cystica and its common sequels, the various types of giant cell tumors. I believe they belong in one series. The evidence for this belief has been accumulating for many years. Barrie, who used to urge acceptance of the inflammatory nature of these tumors, expressed the view many years ago that the whole process is the result of abnormal metabolism of calcium, and this view has been expressed repeatedly since. I did not think it had become convincing until Barr wrote his article bringing in the evidence from all sources, which was very impressive. Since giant cell tumors and osteitis fibrosa are frequently associated with tumors of the parathyroid glands and since various attempts to reproduce the lesion of Recklinghausen's disease by giving parathyroid extract have been successful, one has a body of evidence which to my mind is overwhelming.

As for the assay of parathyroid hormone in giant cell tumors, I doubt if one is justified in expecting a concentration of this hormone in the tumor. Is it not logical to assume that the hormone merely causes a mobilization of calcium from the bones, whereas the tumor reaction is a secondary process resulting from other factors?

ROENTGENOLOGIC ASPECTS. RAYMOND W. LEWIS (by invitation).

There is nothing more difficult in roentgenology than the diagnosis of bone tumors. Although this paper discusses the diagnosis only from the roentgenologic

side, the roentgenologist customarily attempts to arrive at a correct diagnosis only after consideration of all the clinical and laboratory data.

Osteoma, osteochondroma and exostosis are exceptional in that they are usually recognizable at a glance. They are seen most frequently in young persons, near the ends of long bones, and they are composed of varying amounts of bone and cartilage. The bone is of normal structure; the apex is often expanded, and is composed of cartilage with varying degrees of irregular calcification. These growths may be single or multiple, as in the condition sometimes designated "congenital multiple familial exostoses." Occasionally myositis ossificans in appearance simulates osteochondroma. Osteochondroma may become malignant.

Chondroma is prone to occur in young persons. It is most frequently found in the bones of the hands and feet, where it presents a characteristic appearance: a central rarefaction with smooth outlines, sometimes containing scattered irregular calcifications, and an expanded, thinned cortex. It may be mistaken for a cyst or a giant cell tumor.

When chondroma occurs in other bones, its recognition is difficult. Geschickter and Copeland find that the majority of cases of chondroma of the larger type occur about the sternum or costochondral junctions. In long bones the occurrence of this type is rare. Here it expands and thins the cortex and presents a multilocular appearance. It resembles very closely osteitis fibrosa cystica or a slow growing giant cell tumor. Chondroma is prone to become malignant.

Localized osteitis fibrosa cystica, frequently called solitary bone cyst, is usually found before the third decade of life. The most common locations are near the upper extremities of the shafts of the humerus, femur and tibia. The cyst may be unilocular or multilocular. It may show recent or healed pathologic fractures or bowing. It must be differentiated from central chondroma and giant cell tumor. The giant cell variant of bone cyst presents many similarities to giant cell tumor. The cyst must also be differentiated from Brodie's abscess, plasma cell myeloma and carcinomatous metastasis.

Giant cell tumor is apt to be found in the third decade of life or later. Its locations are the ends of long bones, the epiphysis and most frequently the lower end of the femur, the upper end of the tibia and the lower extremity of the radius. Early, the position is asymmetrical and subcortical, and the tumor may show no trabeculae. Later, it occupies the entire end of the bone, and may show no trabeculae. The cortex is expanded, thinned and eventually broken through, with spread into the soft tissues. The lesion very seldom breaks into the joint. The tumor is bone-destroying, never bone-producing, and its outline is always smooth and regular. Lesions of this type must be differentiated from bone cysts, cartilage tumors and from osteolytic types of osteogenic sarcoma. (Slides illustrating the paper were shown.)

DISCUSSION

MAURICE M. POMERANZ (by invitation): Every once in a while I feel that the subject of bone tumors is being overdone, and this sentiment is somewhat intensified after some fortunate diagnosis. When the reverse happens, however, I become convinced that there is much that the roentgenologist might learn regarding these lesions. I am more firmly convinced of it when I consider the interpretations of the films of many of the patients who are referred to orthopedic hospitals.

The roentgenologist fills an extremely important post in an institution where these cases are common, and it behooves him to be thoroughly familiar with his subject. Correct diagnosis must be predicated on knowledge of the subject and an intelligent approach in the interpretation of such patterns as characterize specific tumors. The numerous classifications which have been published are extremely useful for educational purposes, but they lack simplicity. Thus correct interpretation also becomes a clinical problem in which the physical examination and collateral laboratory data are of extreme importance. Fortunately, however, the diagnosis of benign tumors is relatively simple. It is the primary malignant tumor which presents the real problem.

Dr. Lewis has succinctly described the characteristics of benign tumors, and I need not repeat what has been said. I should like to call your attention to common errors in diagnosis which one sees in cases in which the patients are sent to orthopedic hospitals.

You are all familiar with the benign cyst which occurs frequently in the proximal extremity of the humerus. It is often discovered accidentally following some trivial injury and is likely to show a break through one surface of the cortex. The cyst heals following fracture, but one frequently has cases in which the patient is referred for amputation of a limb on the mistaken diagnosis of a malignant tumor. In such instances, the fracture line has been interpreted as a perforation of the cortex, and for some reason or other perforation of the cortex has been considered evidence of a malignant tumor.

It is surprising that such a simple tumor as osteochondroma is often misinterpreted. Recently I reviewed a plate showing a tumor at the lower end of the femur in a man. This lesion was diagnosed elsewhere as an osteochondroma. Yet the film clearly showed periosteal stripping above the lesion as well as some splitting of the femoral cortex. These changes do not occur in osteochondroma, and the roentgenologist should have suspected that he was dealing with a malignant tumor. These small points determine the accuracy of the interpretation. Frequently one roentgenographs small joints following injury and discovers an osteochondroma. In many instances operations have been advised for the removal of such a tumor without inspection of the rest of the skeleton. As osteochondroma is often multiple, attempts at local excision are futile. On the other hand, it may be necessary to remove such tumors if they impinge on important structures such as the brain and pelvic organs, or if they seriously interfere with function, as in the hip joint.

I know of no method by which one can positively identify on the roentgenogram a central chondroma in the shaft of a long bone. In many instances, such a tumor is expansile and trabeculated, resembling in this regard the typical picture of giant cell tumor. As a rule, it is identified by the pathologist following biopsy. Small enchondromas occurring in the small bones of the hands and feet present fairly typical pictures. Occasionally they are confused with sarcoid, but in the latter instance the lesions are smaller, and there are fusiform soft tissue swellings and associated pulmonary changes.

The giant cell tumor is perhaps the most important benign central lesion which the roentgenologist sees. As Dr. Lewis has stated, it is an expansile trabeculated tumor which occurs as a rule in the end of a long bone. Since the original description of this tumor, it has been fairly universally accepted as a class of benign central neoplasm of bone, despite the fact that many of those originally reported subsequently formed fatal metastases. Patients with giant cell tumor are referred to the roentgenologist for either treatment or diagnosis. Although it is not my function here to discuss treatment, I wish to caution you regarding one phase of it. If no typical recalcification occurs following at most two series of treatments with the usual radiation, you are probably not dealing with a giant cell tumor. It may be a chondroma, a chondrosarcoma or a single myeloma. From the standpoint of diagnosis, it is important to remember that other tumors which resemble giant cell tumor may occur in the ends of long bones. In such localities, I have seen single myeloma, chondroma and metastatic lesions from the thyroid, prostate and lungs. In other words, trabeculated lesions in the ends of long bones may be either benign or malignant. It is important to remember this, for one may easily overemphasize the statement that "a trabeculated lesion in the condyle of a long bone is almost invariably a giant cell tumor."

CLINICAL AND THERAPEUTIC ASPECTS. BRADLEY L. COLEY (by invitation).

Benign bone tumors are characterized by slow rates of growth, absence of severe symptoms and a prolonged course; the swelling is not tender, and edema and infiltration of overlying soft parts are generally lacking.

Conditions Due to Dysplasias of Cartilage.—Cartilaginous exostosis, or osteochondroma, is single or multiple. The multiple form commonly affects the ends of the long bones and in particular the upper part of the tibia, lower part of the femur, upper part of the humerus and lower part of the radius. The solitary lesion may occur near the extremity of any long bone, but in my experience the upper part of the humerus has been the most common site. The treatment is surgical removal, but is indicated only when symptoms of pressure are present or when deformity exists. Care should be taken to include a zone of healthy bone at the base and to leave no fragments which may initiate independent growth.

The central chondroma is usually solitary but, as other bones may be affected, roentgenograms of the skeleton are indicated. The central chondroma may simulate a cyst. It is not infrequently found in a phalanx. Pain is usually slight, and when severe, it should arouse one's suspicion that secondary chondrosarcomatous changes have taken place.

The treatment for accessible lesions is removal by thorough curettage and cauterization. Irradiation may be employed for those which, because of their size and location, are inoperable.

Conditions Due to Osteoclasis—Bone Cyst and Giant Cell Tumor.—Bone cyst is one of the more common benign lesions of bone, is frequently mistaken for giant cell tumor, and is occasionally mistaken for malignant tumor. Pain is inconstant, often absent, and half the cases are unrecognized until a pathologic fracture occurs, usually as a result of some trivial injury or muscular effort.

Treatment is conservative. Fracture frequently is followed by healing of the process, though subsequent fractures may occur because this healing phase has not progressed to completion. Successive fractures usually result in shortening of the limb. Operative treatment consists in thorough curettage and cauterization of the cavity with zinc chloride solution or phenol and alcohol, followed by closure of the wound in layers without drainage.

Roentgen therapy has been used, but its results are less certain than those obtained by operation, and there is as yet no certainty that irradiation of a lesion occurring near the epiphysis is free from danger to the growing bone. When cysts occur in flat bones or in the spine, irradiation is indicated.

The so-called giant cell variant of bone cyst is primarily a bone cyst and responds to appropriate treatment as described.

Giant Cell Tumor.—The symptoms are pain, swelling and disability. Owing to its proximity to the joint, the condition is often mistaken for arthritis and treated for a protracted period before roentgenograms disclose its true nature. Swelling is not an early symptom, and the diagnosis should be made before it is manifest. Aspiration biopsy may lead to a correct diagnosis.

Pathologic fracture is a serious complication in giant cell tumor, for it usually leads to an imperfect functional result. Particular care is necessary to avoid this complication when radiation therapy is used for ambulatory patients. Splinting and protection during the healing phase are of great importance.

Treatment is either surgical or by irradiation with roentgen rays. Permanent cures can be obtained by both methods. For tumors occurring in the skull, spine, pelvis and ribs irradiation is the method of choice. For accessible tumors of the long bones of the extremities, especially those of the femur and tibia, surgical intervention compares favorably with irradiation; the period of treatment and disability is shortened, and the functional result is often appreciably more satisfactory.

However, if operation is contemplated, it should be performed by one familiar with the necessity for thorough curettage, cauterization with an escharotic and closure of the wound in layers without drainage. By this technic my associates and I have treated giant cell tumors in fourteen consecutive cases, with no complications and with satisfactory results in each instance.

DISCUSSION

WILLIAM B. COLEY: In discussing the treatment of giant cell tumors of the long bones, I should like to call attention to a statement which I made in 1924

(Coley, W. B.: *Ann. Surg.* **79**:321 and 561, 1924): "The treatment of giant-cell sarcoma of the long bones by radium or x-rays is at present in an experimental stage, and the results thus far obtained do not warrant us in giving up the standard method of surgical treatment (curettage and chemical cauterization) with or without the subsequent use of radium or toxins."

The treatment by irradiation can no longer be regarded as in an experimental stage. During the last few years there has been a marked trend, at least in this country and in France, toward treating all giant cell tumors by irradiation rather than by surgical operation. Practically all of the patients with giant cell tumors of the long bones admitted to the Memorial Hospital for the Treatment of Cancer and Allied Diseases since 1921 have been treated by irradiation.

As recently as 1931, at the annual meeting of the American Roentgen Ray Society in Atlantic City, N. J., the question was raised by a distinguished roentgenologist from Montreal whether giant cell tumors should ever be treated surgically. In replying, a distinguished pathologist of this city stated most emphatically that the surgeon should never be called in to treat these tumors. He added: "I have seen so many bad results from recurrence, malignant transformation, infection of all types, acute and chronic, resulting in amputation, that I have no longer any room to file these cases in our museum."

Our group of giant cell tumors of the long bones treated by surgical methods practically equals in number the group treated by irradiation, since Dr. Bradley Coley and I have continued to employ surgical methods in all of our private cases at the Memorial Hospital and in all cases at the hospital of the New York Society for the Relief of Ruptured and Crippled. A careful study of our series reveals the fact that most of the bad surgical results occurred a decade or more ago and in the hands of surgeons who had had little experience in the treatment of bone tumors. There has not been a single bad result following curettage at the Memorial Hospital since the Bone Sarcoma Department was established there nearly twenty years ago. The only bad surgical result in our series occurred in a case of very extensive giant cell tumor of the fibula in which, with the assistance of Dr. J. P. Hoguet, I performed a total excision in 1918. Unwisely, I fulgurated the extensive wound with the Keating-Hart apparatus, leaving a surface of charred muscle. Gas gangrene occurred, necessitating an immediate amputation. The patient made a good recovery and was well when last traced some ten years later. This was a very rapidly growing tumor, clinically malignant (microscopically, a giant cell tumor), and I am certain that amputation would have been the method advised by most of the surgeons at that time. In 1917 I had a case in which a giant cell tumor of the femur operated on at the hospital of the New York Society for the Relief of Ruptured and Crippled was followed by a serious infection requiring amputation of the leg.

On the other hand, the bad results following irradiation, although rarely mentioned, are, I believe, more numerous and far more serious than the bad results of surgical intervention which are so often emphasized. Among these are stiff joints, severe burns and late ulceration. In 1 case of giant cell tumor at Memorial Hospital in which treatment was by irradiation, osteomyelitis of the femur developed eight and a half years later. An attempt was made to save the limb by conservative operation, but after another year of complete disability of the patient, the wound remaining unhealed, it was found necessary to perform an amputation nine and a half years after the beginning of treatment.

A comparison of the results obtained in a group of 54 cases of giant cell tumor in which the involved tissue was treated by irradiation at the Memorial Hospital with an almost similar number treated by surgical methods warrants the opinion that there is still a place for surgical operation in the treatment of giant cell tumor; in fact, I believe it should occupy the first place.

My conclusions reached after a careful study of this series of 100 cases of so-called benign giant cell tumors of the long bones are:

Primary amputation should practically never be performed for giant cell tumor of a long bone.

Primary curettage with chemical cauterization (50 per cent zinc chloride) without packing or drainage should be regarded as the method of choice, for the following reasons: (*a*) It eliminates the error in diagnosis which is associated with treatment by irradiation without biopsy—the method advocated by Ewing and Herendeen and very widely adopted at present. This error occurs in 20 per cent of the cases in which that treatment is used. (*b*) The period of inability following surgical treatment is markedly shorter than that following irradiation. (*c*) The function of the joint is much better following surgical treatment. (*d*) The number of cases in which pathologic fracture requires secondary amputation of the limb is much smaller than when primary irradiation is employed. (*e*) The number of cases in which the tumor later becomes malignant osteogenic sarcoma is smaller.

An open incision to take a specimen for biopsy should never be performed in a case of giant cell tumor of a long bone since without curettage it is very likely to be followed by formation of a sinus and infection.

Regarding the use of erysipelas and prodigiosus toxins: While a number of far advanced and some hopeless inoperable giant cell tumors have been cured by toxins alone, I believe that surgical curettage, as just described, is the method of choice for the operable ones. The period of disability is shorter, and the certainty of cure is greater. I believe that the use of toxins for two or three months as a prophylactic in all cases of advanced giant cell tumor of a long bone will greatly lessen the number of recurrences following curettage (Bloodgood's series, in which the toxins were not used, showed 30 per cent recurrences). The toxins should be used (injected directly into the tumor) in all cases of inoperable giant cell tumor, especially of the spine and pelvic bones.

(Lantern slides showed in detail the results obtained with the various methods of treatment.)

In closing, I should like to say a word about the influence of trauma on the development of giant cell tumor. I believe that local trauma is the most important of all causative factors. In our own series there has been a history of antecedent local injury in 56 per cent of the cases, and in the Johns Hopkins series reported by Geschickter and Copeland, in 65 per cent. These percentages cannot be explained away on the ground of coincidence. In several of our cases in which apparent cure of a giant cell tumor of a long bone had occurred, an actively growing recurrent tumor followed almost immediately after a new local trauma. These cases, in my opinion, come very near to affording a scientific demonstration of a causal relationship. Geschickter and Copeland, in their book on bone tumors (Geschickter, C. F., and Copeland, M. M.: Tumors of Bone, New York, American Journal of Cancer, 1932), state: "We are in accord with these authors (German authorities) in placing trauma in a primary position in the production of giant cell tumors and bone cysts." Furthermore, they believe that a series of x-ray pictures taken at frequent intervals from the time of the trauma through the stages of hemorrhage, bone destruction and replacement with tumor tissue confirms this view and furnishes strong proof that the trauma was a causative factor.

BRADLEY L. COLEY: It is my belief that the question of whether giant cell tumor should be regarded solely as a problem for the roentgenologist has not been finally decided. Operative treatment, however, should not be undertaken without special experience and training in this field of surgery. The end-results of surgical treatment have in our experience compared favorably with those of irradiation in specially selected cases in which we believed this form of treatment indicated.

Book Reviews

The Brain as an Organ: Its Postmortem Study and Interpretation. By Frederic and Florence Wertham. With an introduction by Adolph Meyer, M.D., Johns Hopkins Hospital. Price, $7.50. Pp. 538, with over 160 illustrations. New York: The Macmillan Company, 1934.

The book begins with a consideration of the importance and need of studying the brain as a whole, which means all its parts and all the elements of its structure. The tendencies to correlate symptoms with structural changes of doubtful significance are condemned. "It is the nosological urge, coupled with an undue emphasis on microhistological details demonstrable by selective staining methods that has made histopathology [of the central nervous system] such an involved and complicated subject." A new principle is needed to guide the study of lesions of the brain, namely, the conception of the brain as an organic entity. In diseases of the brain other organs must not be neglected in the examination, and in somatic diseases the brain must not be neglected. These are sound principles to which pathologists in general will subscribe unhesitatingly.

The second section describes the macroscopic examination and dissection of the brain and cord. The method developed by the authors in the course of their own work is given in detail; it will be found helpful to pathologists who strive to make the best possible use of their brain material. The third part, which contains eighty-four pages, deals with the principles and methods of histologic technic, including fixation, embedding and sectioning, as well as staining methods. This outline with its "survey methods," "primary analytic methods" and "supplementary methods" for special problems will be of great value to all who are concerned with the histologic examination of nervous tissues and its results.

The headings of the succeeding ten sections give a good idea of the rest of the book: "Alterations of the Individual Components of the Nervous Tissue (Nerve Cells, Nerve Fibers, Glia, Ground Substance, Blood-Vessels, Amorphous Substances)"; "Kinds of Lesions"; "Distribution of Lesions"; "Extent of the Normal"; "Interpretation of Lesions"; "Correlation of Lesions with Psychopathological Phenomena"; "Comparative Histopathology"; "Some Histopathological Syndromes That May Be Associated with Mental Disorder"; "Is There a Histopathology of Schizophrenia?" and "Forensic Neurohistology."

There are a full index and a selected bibliography of one hundred and seventeen references. The paper is good and unglazed. On separate pages at the end are one hundred and sixty-six excellent illustrations reproduced by the "aquatone process" from drawings by Florence Wertham. These illustrations help much in understanding the structural changes described in the text.

The pathologist who undertakes experimental work on the brain will find the section on the comparative histopathology of the brain of special interest; here again the brain is dealt with as an organ.

Certain views and considerations are developed in this book that merit special mention. In the section dealing with the extent of the normal many conditions currently regarded as abnormal are shown to be relatively of little significance. The modern ideas of the functional disorders of blood vessels are presented clearly. This presentation of views that are opposed to mechanical conceptions will be of special interest to the general pathologist. Probably there is no structural change that is absolutely characteristic of any single condition, and conclusions based on the study of a limited number of one kind of nerve cells may not be applicable to nerve cells in general. The brain reacts in similar ways to various kinds of damage, and these reactions are limited in scope; the versatility of the symptoms of nervous diseases is not paralleled by the structural changes. Various stages in the same process may show

different changes: "The microscopic picture is not one of fixed structure; rather it is an instantaneous photograph that catches one scene only from a whole dynamic series of events." Under the influence of the conception of functional localization the distribution of lesions in various parts of the brain has been neglected. The central nervous system must be studied as a whole if the structural changes are to be understood correctly. As examples of a much wider distribution of lesions than was first announced may be mentioned epidemic poliomyelitis, hereditary ataxia, paralytic dementia, tumors and forms of meningitis and encephalitis. There may be profound disturbances of mental function without demonstrable structural changes: "There are cases of idiocy of the severest type in which no significant histological lesions may be demonstrable in the brain." The difficulties of correlating neuropsychiatric symptoms with structural changes are stressed repeatedly, and it is pointed out that all attempts to find in the brain some region or regions specific for psychosis have flatly failed. Even such distinctive lesions as those of paralytic dementia may occur under totally different biologic circumstances. Schizophrenia has not been established as an organic disease of the brain.

The histopathologic syndromes that may be associated with mental disorder form a heterogeneous group which includes atelokinetic disorders (agyria, pachygyria, microgyria); tuberous sclerosis, syringomyelia, Recklinghausen's disease; amaurotic family idiocy, which is part of a general disorder of lipoid metabolism; diffuse sclerosis; meningitis; encephalitis; hepaticolenticular degeneration; alcoholic disease of the brain; paralytic dementia; cerebrospinal syphilis; cerebral arteriosclerosis; chronic progressive hereditary chorea; circumscribed cortical atrophy; senile dementia and Alzheimer's disease. Reading this chapter (11) alone will stimulate the pathologist to give increased care to his provisions for examination of the brain.

The final section concerns the medicolegal bearings of lesions in the central nervous system. The necessity of competent thorough examination of the nervous system in medicolegal cases is self-evident. In no other field of pathologic anatomy does the work of the coroner's office in this country fail so completely. In carbon monoxide poisoning, epidemic encephalitis, alcoholic disease of the brain, syphilis and trauma there may be lesions of the brain of diagnostic value, but, on the other hand, it is not possible to establish forensically on the basis of structural changes in the brain that a person suffered from epilepsy or psychosis.

In his introduction Adolf Meyer praises the book because of its "constructively critical evaluation of the methods, their results and interpretation and its wholesome perspective." This praise is merited. The book will be of great service to pathologists, as well as to neurologists and psychiatrists. There is no other work that deals with the structural changes in the brain in a fashion so constructively critical, comprehensive and altogether competent.

Books Received

STUDIES ON CELL GROWTH: II. THE GROWTH IN VITRO OF NORMAL MOUSE CELLS AND OF MOUSE CANCER CELLS (CARCINOMA SARCOMA) IN NEUTRAL AND IMMUNE MEDIA (SERUM PLASMA). M. J. A. des Ligneris, M.D. (Bern), L.M.S.S.A. (Lond.). Publications of the South African Institute for Medical Research. Edited by the Director. No. XXXIV, volume VI, pp. 313-322. Johannesburg: The South African Institute for Medical Research, 1934.

THE CLINICAL ASPECTS OF VISCERAL NEUROLOGY WITH SPECIAL REFERENCE TO THE SURGERY OF THE SYMPATHETIC NERVOUS SYSTEM. W. K. Livingston, M.D., Clinical Associate in Surgery, University of Oregon Medical School. Price, $5. Pp. 254, with 46 illustrations and 3 color plates. Springfield, Ill.: Charles C. Thomas, Publisher, 1935.

ANNUAL REPORT OF THE INSTITUTE FOR MEDICAL RESEARCH FOR THE YEAR 1933. P. H. Martin, Acting Director, Institute for Medical Research, Kuala Lumpur, Federated Malay States. Pp. 143. Kuala Lumpur: Federated Malay States Government Press, 1934.

ANNUAL REPORT OF THE SURGEON GENERAL OF THE PUBLIC HEALTH SERVICE OF THE UNITED STATES FOR THE FISCAL YEAR 1934. Cloth. Price, 75 cents. Pp. 143. Washington, D. C.: United States Government Printing Office, 1934.